FLIPR^{3}®

Fluorometric Imaging Plate Reader

Operator's Manual

Molecular Devices

PN 0112-0082 Rev. A

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System Overview 1

Introduction

Chapter Contents

This chapter provides an overview of the FLIPR® system and components.

Overview

Introduction

The FLIPR systems are fluorescence-based microplate readers with an integrated 384- or 96-well pipettor. They perform rapid throughput cell-based assays and provide drug discovery scientists accurate and precise kinetic data. They are used in many types of assays, such as those designed to monitor changes in membrane potential, intracellular Ca^{++} flux, and intracellular pH.

System Description

The FLIPR system's basic principle of operation is to simultaneously illuminate the bottom of a 384- or 96-well plate and measure the emitted fluorescence from all of the wells using a cooled CCD camera. The cell plate wells contain cells loaded with a fluorescent dye whose emission characteristics change upon binding with a particular ion (*e.g.*, Ca^{++}, H^{+} or Na^{+}). The cell plate has a clear bottom (to allow excitation and signal access) and black walls (to prevent signal diffraction). Cells, which can be adherent or non-adherent, must be at the bottom of the well.

The FLIPR system consists of the following components:

- Versatile 6-position platform
- Integrated 384- or 96-well pipettor
- Excitation and emission optics
- Argon-ion laser
- Custom software to control instrument parameters and to acquire and transfer data to spreadsheet-compatible data files

For each individual cell plate, up to three compound plates can be used. The FLIPR system can be programmed to take multiple time-point exposures of the cell plate, recording the fluorescence signal over time at intervals determined through the system control software. The compounds are pipetted into the cell plate, while the signal emitted by the cells is collected by the CCD camera.

The FLIPR system control software displays the fluorescence data for all 384- or 96-wells on the computer's monitor. Data updates occur in real-time. Once the run is completed, you can export the data for post-run analysis. The data can be converted to a fluorescence signal as a function of time (time sequence), or it can be converted to one number per well (statistic). The data is stored in an ASCII text file format for input into a spreadsheet or data-base program.

The following subsections provide brief descriptions of the principal system components and features. Detailed explanations of the hardware components are found in Chapter 2, *"System Hardware Features."*

System Components

The FLIPR System Tower

The FLIPR system tower houses the components described in the following subsections. All of these components are accessed via a vertical sliding door.

The Six-position Platform

The pipettor can access six positions on the system platform. Two positions are reserved for tip loading and washing. The configuration of the remaining four positions is determined by the addition of one of three optional stages: simple,

heated, or stacker stage. The choice of stage depends on the type of assay format. In all cases, the front left position is reserved for the read plate (either a 384- or 96-well plate).

The simple stage allows you to run the system manually or integrated to a robot without temperature control. For each experiment, one to three compound plates (either 384- or 96-well), one cell plate, and one tip rack are placed on the stage manually or by a robot integrated into the FLIPR system. The tips are automatically loaded by the pipettor. A single set of tips can be used for multiple cell plates by programming the tips to be automatically washed between runs.

The heated stage provides temperature control for the cell plate and one compound plate. This stage also regulates the temperature for two additional compound plates.

The stacker stage allows you to automate a large run. Cell and compound plates can be stored in a stacker located outside the instrument. Magazines of 50 plates may be used. One cell plate and one compound plate can be shuttled into the FLIPR system per run. Two fixed compound plates may also be used.

The 384-/96-well Pipettor

The pipettor allows you to simultaneously add compounds to all of the wells of the cell plate. The system can be fitted with either a 384- or a 96-well pipettor head. If the system is fitted with a 96-well pipettor head, you may use both 96- and 384-well compound plates. The 96-well pipettor can dispense fluid in quadrants: four 96-well additions per 384-well cell plate. Deep-well plates or reservoirs can be substituted for standard 96- and 384-well plates.

! WARNING ! Do NOT attempt to change plate positions or add additional plates when the pipettor is in motion. The door to the system platform should remain closed during normal system operation. Do not operate the instrument if the door does not close before the beginning of a run.

The Excitation/Emission Optics

The excitation optics provide complete illumination of the cell plate, while the emission optics allow the CCD camera to simultaneously capture the emitted signal from the entire plate. The typical update frequency for Ca^{++} assays is every second. A 510–570 nm bandpass emission filter located in front of the camera lens blocks the excitation light and defines the detection range.

The Argon-ion Laser

The FLIPR system is supplied with a 5W argon-ion laser source. The standard excitation wavelength used in the FLIPR system is 488 nm. Excitation wavelength can be adjusted to 514 nm with instruction from a field service engineer.

The Plate Stacker and Washer

The stacker and washer are stationed over a shroud covering the laser. A diving board transports cell/compound plates from the stacker to the instrument, then back in the stacker waste magazine. The stacker can also shuttle cell plates to the washer prior to the assay. The washer accommodates 96- and 384-well plates.

The Host Computer

The computer system is described in Chapter 2, *"System Hardware Features."*

System Requirements

About System Requirements

This section lists the electrical, physical and environmental requirements of the FLIPR system. Please refer to the *FLIPR Pre-Installation Manual* for more details.

Electrical

A single 110 VAC or 220 VAC 15A room outlet is required to power all of the system components, except the laser. A 220 VAC, 50 Amp, 3-phase power source with disconnect is required for the laser. A separate transformer must be purchased from Coherent in order to properly condition the 3-phase power for the laser.

In the interests of both safety and performance, the electrical service for the laser must be configured so that all three phases are symmetric with respect to the building ground. The measured voltage between phases should be 208 VAC ±10%. The voltage between each phase and building ground should be approximately 120 VAC. The service should also have a main power disconnect in the same room as the laser. All of the electrical preparations for laser power are to be completed by qualified electricians.

Physical

Laser Cooling

There are three approved methods of cooling the argon-ion laser:

- Direct house water supply with a minimum flow rate of 2.5 gal/min.
- A water-to-water heat exchanger
- A closed-loop chiller
- Direct house water is not an appropriate choice if the water temperature can drop below 10° C in winter. Most users employ a heat exchanger or a chiller.

More information about laser cooling is available in the *FLIPR Pre-Installation Manual* and in Coherent's documentation for the laser.

Minimum Space

The optical table provided with the FLIPR system is 96 inches long and 30 inches wide. You will need a minimum of 24 inches at the right side of the instrument for the camera and filter access, and 24 inches behind the system in order to perform maintenance. The minimum room dimensions are 10 feet x 8 feet. The table can be supplied with optional wheels so it can be moved to make necessary adjustments and perform maintenance.

! WARNING ! The table top can weigh as much as 250 lbs (114 kgs). If a forklift or hoist is not available, ensure that adequate personnel are present when installing or moving the table. Follow all necessary safety precautions and use proper lifting techniques.

Air

The FLIPR system requires an air source that can produce a steady supply of clean air at 80 psi. House air may be used if it is regulated and reliable. You may use a nitrogen tank with a two-stage regulator or compressed air.

System Hardware Features

2

Introduction

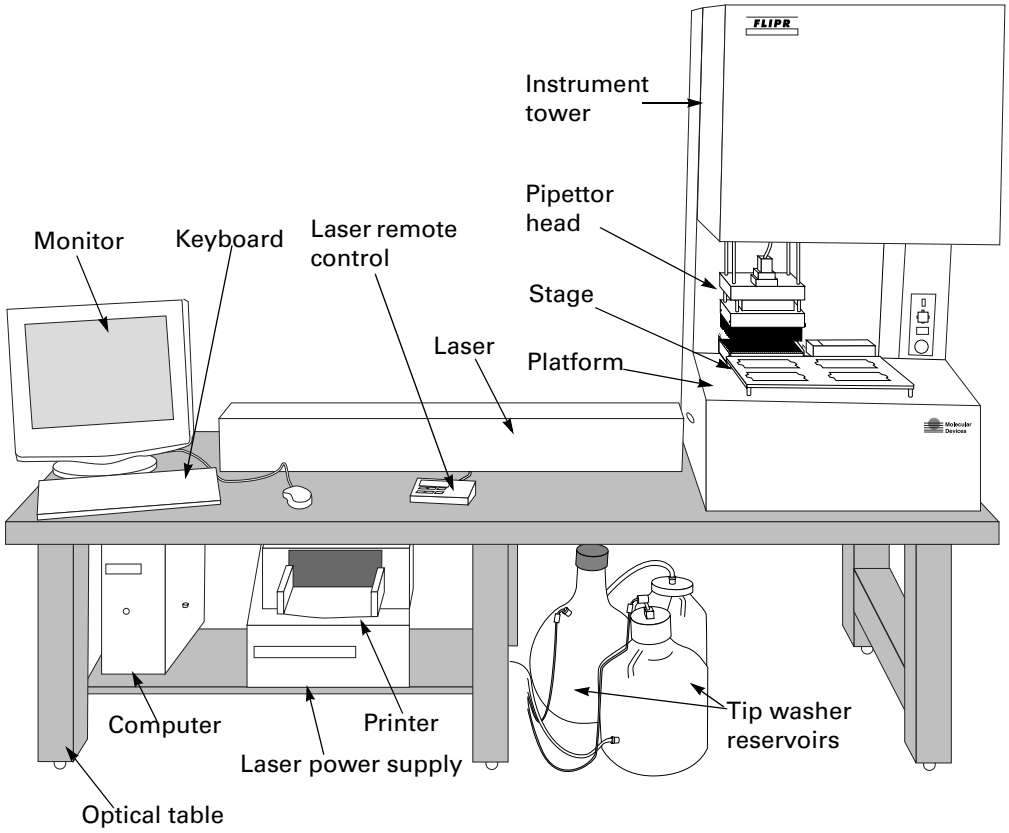
Chapter Contents

This chapter provides information regarding the hardware features of FLIPR®.

Overview of the System Hardware Features

System Diagram

This chapter provides brief descriptions of the hardware features available with the FLIPR system. These features are shown below.

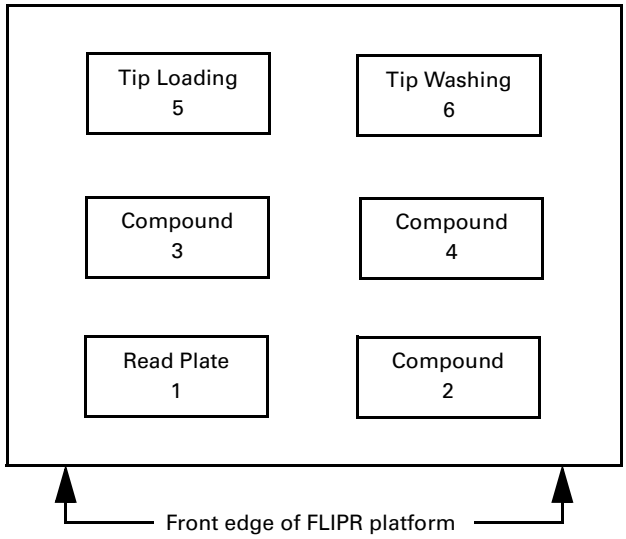


Features of the Plate Handling System

Six-position Platform

The 96- and 384-well plates are placed in the FLIPR system's 6-position platform (shown below) according to the following pattern:

- 1 read position (Position 1)
- 3 compound plate positions (Positions 2, 3 or 4)
- 1 automatic tip-loading position (Position 5)
- 1 automatic tip-washing position (Position 6)



Positions 5 and 6 are devoted to tip loading and washing, respectively. Position 1 is reserved for the cell plate. The remaining positions (2 through 4) hold the compound addition plates and are configured in three possible ways, depending on the type of stage. The available stage types are:

- Simple stage
- Heated stage
- Stacker stage

Each stage is installed on the instrument's platform in the same way. The stage has four "feet." The front two feet have locating pins that protrude and fit into corresponding holes in the platform.

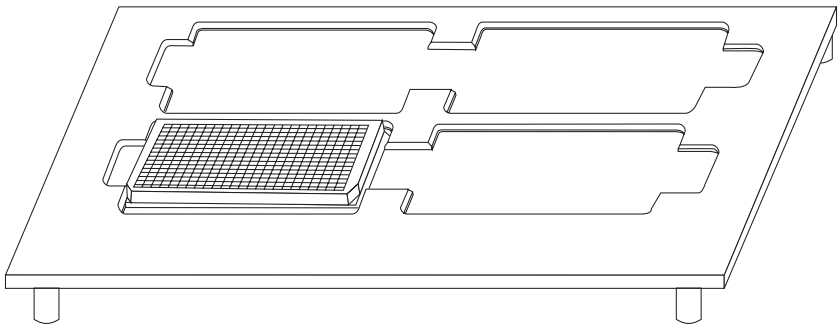
Once the stage is installed, the software has to recognize the installed stage. Select **Hardware** from the **Setup** menu and select the appropriate stage-type.

Note Assays cannot be run without one of these three stages installed on the instrument's platform.

Simple Stage

This stage is used for manual and robot-integrated operation of the FLIPR system and has four defined positions for microplates. All plates and tips are positioned by the user or the robot. One position (Position 1) is assigned to the read plate, and the remaining three positions are assigned to compound plates. Up to three compound plates can be used in a single assay.

Simple Stage



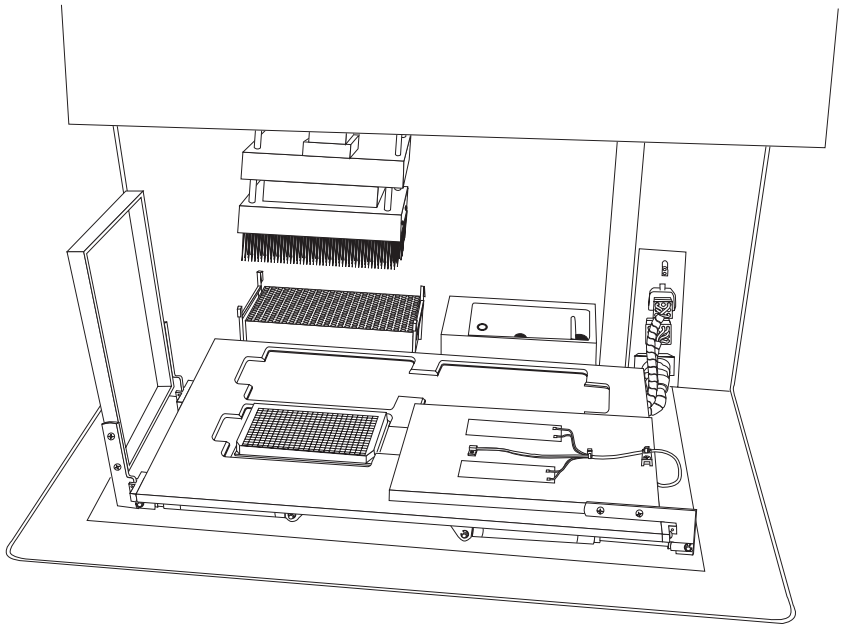
If a 384-well cell plate is used, Position 1 fitted with an open mask and cell plate rests on a removable ledge surrounding the opening. For instructions on installing the ledge, refer to Chapter 8, *"Maintenance."*

If a 96-well cell plate is used, Position 1 is fitted with the slit-shaped mask or the ledge supplied with the instrument. For instructions on fitting or changing a mask, refer to Chapter 8, *"Maintenance."*

Heated Stage

The heated stage also contains four positions for microplates. The front two positions (Positions 1 and 2) hold the cell plate and one compound plate, respectively. They are covered by clamshell-type lids. The two rear positions are uncovered (see the figure below).

Heated Stage



In Positions 1 and 2, the temperature is controlled by both the bottom of the stage and the lids. The temperature in Positions 3 and 4 is controlled by the bottom of the stage only. To install the heated stage, position its feet in the holes in the FLIPR platform, then connect its cables to their corresponding sockets at the right rear of the platform.

Once the stage is installed, the software has to recognize the installed stage. Select **Hardware** from the **Setup** menu and select the appropriate stage-type.

Note FLIPR systems provide no humidified air flow.

The clamshell lids over Positions 1 and 2 are opened and closed pneumatically via cylinders located under the stage. The set temperature ranges from ambient temperature (+5° C) to 40° C. The time required for the set temperature to reach equilibrium is approximately 20–30 minutes.

When using temperature control in an assay, the FLIPR system sequentially performs the following operations:

a. Clamshell lids close over Positions 1 and 2.
b. Pipettor picks up the pipette tips from Position 5 and moves to Position 4.
c. Clamshell lid opens over Position 2.
d. Pipettor moves to the compound plate in Position 2.
e. Pipettor picks up fluid and repositions itself over Position 3.
f. Experiment starts and the initial fluorescence is measured.
g. When programmed for fluid addition, the lid over Position 1 opens and the pipettor repositions itself over the read plate and delivers fluid.
h. Pipettor returns to the "Home" position while the clamshell lids close and the experiment continues.

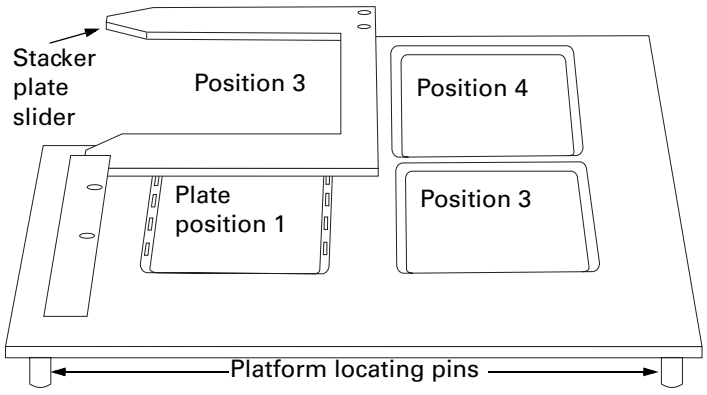
Note The FLIPR system's main door will remain closed for the duration of the experiment.

Note The operations above can be changed, depending on the experimental setup.

Stacker Stage

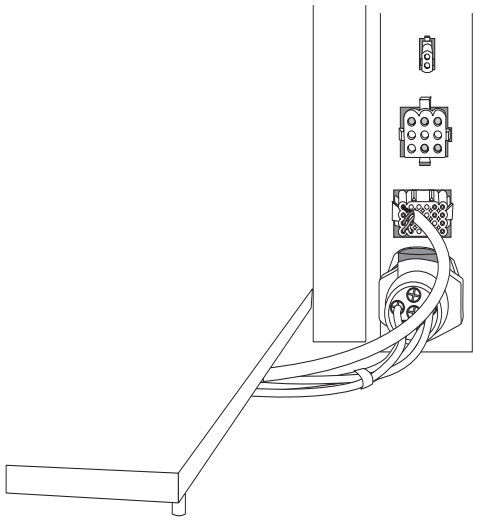
For information on the stacker stage, please see Chapter 6, *"Using The Plate Stacker."*

The stacker stage is comprised of four positions. Cell and compound plates for positions 1 and 3 are fed by a 50-plate stacker, via a conveyor belt. Compound plates for positions 2 and 4 are placed manually on the stage. Compound plates, used in Positions 2 and 4, can be the standard or deep-well variety. Fluid troughs can also be used.



The stacker stage is positioned on the instrument platform in the same manner as the simple and heated stages. Once the stage is in position, connect the stacker stage to the FLIPR instrument by inserting the circular and square connectors into the matching plugs located above the stage.

Once the stage is installed, the software has to recognize the installed stage. Select **Hardware** from the **Setup** menu and select the appropriate stage-type.



The stacker itself rests on a shelf over the top of the laser cover. It is guided by two pins located on the FLIPR system. The stacker's plate magazines should be filled so that cell plates alternate with compound plates. When not using Position 3, you can stack just the cell plates.The stacker stage has a pneumatically-driven shuttle system that moves plates between Positions 1 and 3. This shuttle ensures that cell plates are always placed in Position 1 and compound plates are sent to Position 3.

When using the optional stacker mechanism and its corresponding stage, the FLIPR system will sequentially perform the following operations:

a. The stacker mechanism's conveyor belt brings a cell plate to Position 3 on the instrument platform.
b. The shuttle slides the cell plate to Position 1.
c. The conveyor brings a compound plate to Position 3.
d. The pipettor picks up pipette tips.
e. The pipettor moves to a compound plate and picks up a preprogrammed amount of fluid.
f. The pipettor moves to Position 1 and delivers the fluid to the read plate, as determined by the control software. Additional compounds can be added from other compound plates.
g. The experiment ends and the compound plate is removed from the platform by the conveyor.
h. The compound plate is shuttled to Position 3, removed from the instrument and deposited in the waste plate stack. The read plate follows a similar path.

Note The FLIPR system's main door remains closed for the duration of the experiment. Plates are transported in and out of the instrument before and after the experiment on the conveyor and shuttle system.

Features of the Dispensing System

Integrated 384- or 96-tip Pipettor

The FLIPR system uses an integrated, computer-controlled pipettor that may be configured with either a 384- or 96-tip head (to change the pipettor head, please contact Molecular Devices' Service Department 1-800-635-5577). The pipettor aspirates, dispenses and mixes fluids from up to three compound plates. All operations are controlled by the system control software.

One of the key requirements of the pipettor for drug screening is the ability to perform accurate and precise fluid additions. This ability ensures well-to-well data fidelity. Pipettor accuracy is 3–5% for 25 µL additions.

Unless defined otherwise in the experimental setup, the pipettor automatically loads tips at the beginning of an experiment and unloads them into the rack when the experiment ends.

Note Tips may be washed between experimental runs.

Tip Loading

The pipettor automatically loads either 384 or 96 tips simultaneously from a rack placed in Position 5 on the instrument platform. Black or clear tips can be used.

Position 5 contains the tip rack holder. This holder allows the pipettor head to grasp the rack during tip loading. Depending on the FLIPR system configuration, tip racks may be inserted either by hand or by a robot arm.

CAUTION Make sure that the tip rack is seated in the middle of the carrier.

IMPORTANT Molecular Devices strongly recommends that you use only the tips specified for use with the FLIPR system. Using the correct tips ensures proper seating of the tips on the tip cylinder head and prevents damage to the pipettor. For a list of recommended tips, refer to Appendix A, *"Consumables Used With the FLIPR System."*

Note To ensure proper tip seating, the air source must be a minimum of 80 psi. Recommended maximum air pressure is 90 psi.

IMPORTANT DO NOT touch the tip rack in the holder once the tips are loaded. If the tip rack is dislodged between loading and unloading of the tips, the pipettor will be unable to return the tips to the rack. If moved, you can go to **Run**, **Special Operations** to unload tips.

Tip Washing

The FLIPR pipettor uses disposable tips, but you can choose to wash and re-use them between cell plates. Tip washing between experimental runs is performed at Position 6, which contains a wash basin. Tip washing can be programmed to occur at the end of an experiment or after the last fluid addition or between fluid additions.

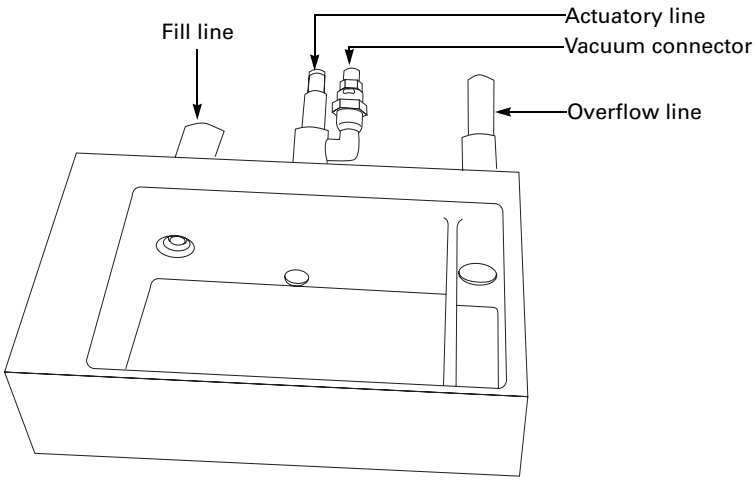
The wash basin is connected to the wash solution supply, a waste reservoir and an overflow reservoir. The containers are usually located below the instrument table.

A peristaltic pump is used to introduce wash solution into the basin. A vacuum pump drains the wash solution after each cycle. Both pumps are controlled by the FLIPR computer.

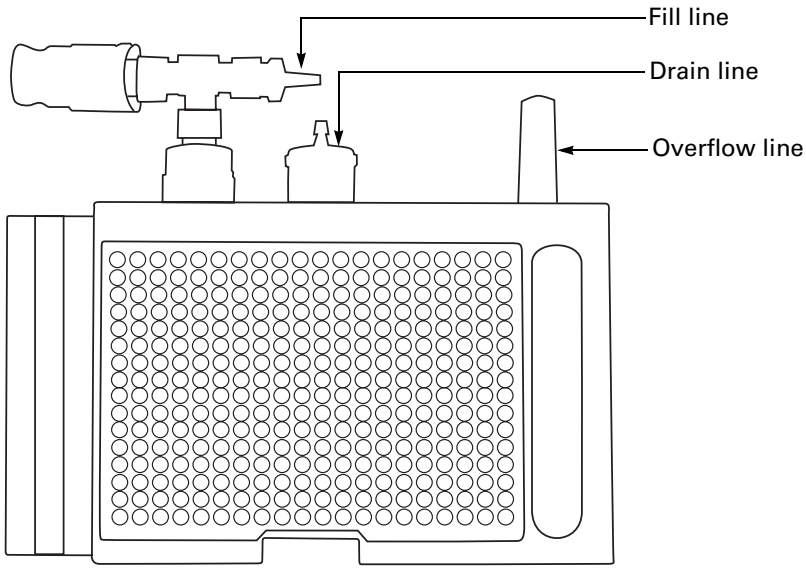
Wash solution enters the basin through an opening in the upper rear side of the basin. The overflow reservoir is located to the right of the wash basin. The bottom of the wash basin has a central drain through which the waste is aspirated at the end of the wash cycle. The wash basins for FLIPR^2 and FLIPR^3 are shown below.

The wash parameters can be controlled through the software.

Wash Basin for FLIPR^2



Wash Basin for FLIPR^3



Features of the Optical System

Excitation Optics

The purpose of the illumination optics is to simultaneously illuminate all 384- or 96-wells in the cell plate with the greatest efficiency. When the light from the laser enters the FLIPR system, it is conditioned by a series of lenses and subsequently projected onto a 20-facet polygonal mirror. The mirror rotates at 18,000 rpm and scans the entire cell plate at the rate of 600 scans per second.

The cell plate is protected from constant illumination and possible dye photo-bleaching by a shutter located immediately below the cell-plate position. When fast camera updates are used (*e.g.*, less than 2 seconds), the shutter opens at the beginning of an experiment and illuminates the plate. The shutter remains open until either the update sequences are slowed or the experiment ends. With slower update times, the shutter closes between exposures.

It is important to note that with the laser source, no excitation filters are required. This would not be the case if a broadband light source were used. However, an emission filter is required.

Emission Filters

A 50 mm diameter interference filter is positioned in front of the camera to separate out the emission band of the dye being used. The filter chosen must both isolate the emission band and reject unwanted excitation light. The most common configuration in the FLIPR system is a laser excitation wavelength of 488 nm used with a 510–570 nm bandpass emission filter.

CCD Camera

The CCD camera is located beneath the cell and compound plate platform.

If you have a FLIPR^2, the camera controller, located on the shelf beneath the plate table, contains most of the electronics that run the camera. There is one switch on the front of the camera controller. It supplies power to the controller and turns on the thermoelectrical camera cooler.

The camera requires 10–20 minutes to reach a cooled operating temperature of –35° C.

! WARNING ! Using the camera before it has reached its operating temperature will result in noisy data. The FLIPR status bar displays the correct camera temperature.

The camera is an integrating-type detector. It must temporally integrate in order to build up the signal-to-noise ratio. Depending on the intensity of the excited light (which depends on dye efficiency and laser power), it may be necessary to utilize longer camera exposures. This will prevent the measured fluorescent signal from being dominated by detector noise. Additional light may also be provided by changing the shutter aperture via the f/stop setting. Decreasing the f/stop value increases the aperture size. The minimum f/stop setting is 1.4. A small door, located on the right side of the instrument and below the level of the platform, allows you to access the aperture setting wheel.

CAUTION For safety reasons, an interlock will activate a shutter, covering the laser beam's entrance into the optical enclosure when the door is open. Under no circumstances should the interlock ever be overridden.

Argon-ion Laser

For a general tutorial on the operation and/or maintenance of the laser, please refer to the Coherent I 90-C laser manual supplied with your system. If you have questions concerning the proper operation of the laser, please call the Molecular Devices Service Department (1-800-635-5577).

! WARNING ! Never remove the laser enclosure while the laser is in operation or try to observe the laser radiation beam directly.

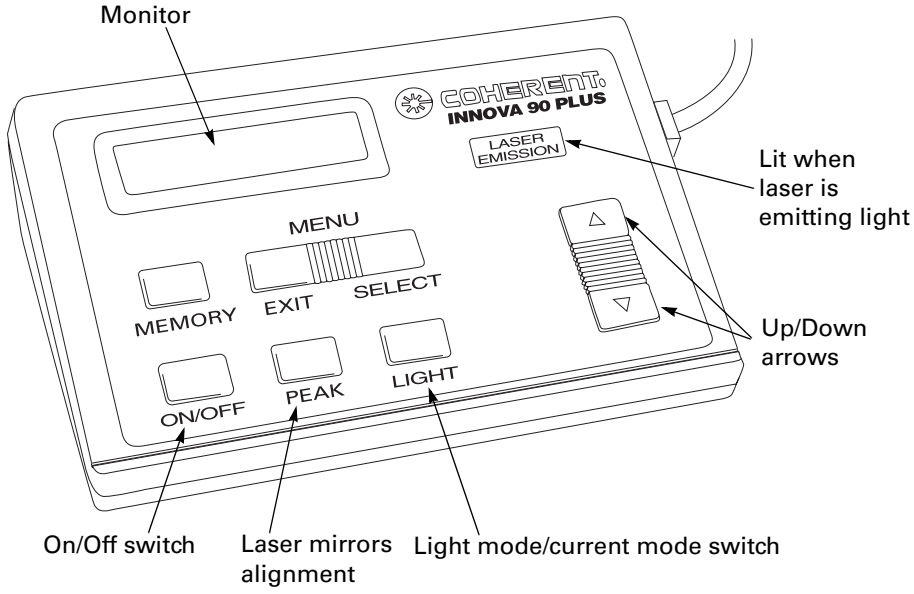
IMPORTANT If you experience problems with the laser, shut down the operation immediately. Refer to the Coherent I 90-C laser manual or contact Molecular Devices before attempting any troubleshooting.

The Innova® 90 Plus series ion laser, used with the FLIPR system, complies with the U. S. government Center for Devices and Radiological Health (CDRH) requirements for laser safety. Under CDRH regulations, the Innova® 90 Plus series ion laser alone is a CLASS IV laser. However, when the laser is integrated with the FLIPR system, it can be considered a CLASS I laser due to the enclosures that surround the beam and prohibit or limit access to the laser radiation (ANSI Z-136.1-1993).

The FLIPR system uses a 5-Watt argon-ion laser to provide the energy to excite specific dyes emitting fluorescence. Any fluorescent dye being used must have visible spectrum excitation characteristics. The standard excitation wavelength is 488 nm. Please call Molecular Devices if you have questions about alternate excitation wavelengths.

The laser's power supply is located beneath the instrument table. The laser is controlled by a remote control attached to the rear of the laser power supply by a 6-foot cable. The remote typically sits on the instrument table in front of the laser. The remote control provides the user with the current input (in Amps) and the light output (in Watts) of the laser.

When the laser is in **Current** mode (**CUR** is displayed on the remote control), the amperage can be set. When the laser is in **Light** mode (**LT** is displayed on the remote control), the wattage can be set. By pressing **SELECT**, you can toggle between the two modes. You can modulate both the amperage and wattage by pressing the **UP/DOWN** arrows on the remote control (see below).



Always run an assay with the laser in Light mode, so that the light output is set to a constant value throughout the assay. Current mode is used to align the laser's mirrors (see Chapter 8 *"Maintenance"* for more information about laser mirror alignment).

For each assay, you must determine the optimum excitation laser power. In general, it is better to use no more laser light than necessary in order to prevent photo-bleaching.

Plate Stacker and Washer

Please refer to Chapter 6, *"Using The Plate Stacker,"* and Chapter 7 *"Using the Plate Washer"* for detailed information.

Features of the Computer System

Computer

The FLIPR system's control software runs in the Windows NT 4.0 environment on Intel processor-based computers. The minimum configuration required is:

- Intel Pentium II© processor (300 MHz or above)
- Windows NT 4.0 OS
- Service Pack 4 or above
- 128 MB of RAM (or above)
- Hard Disk Drive (4 GB or above)
- SCSI controller
- Ethernet interface
- CD-RW drive
- 1024 x 768 monitor
- Inkjet printer (not required but supplied)

A network card and a data backup device have been installed in the FLIPR system. Please contact Molecular Devices Corporation for any specific issues regarding the system's computer.

System Start-up and Shut-down Procedures

3

Introduction

Chapter Contents

This chapter provides the procedures for starting up and shutting down the FLIPR® system.

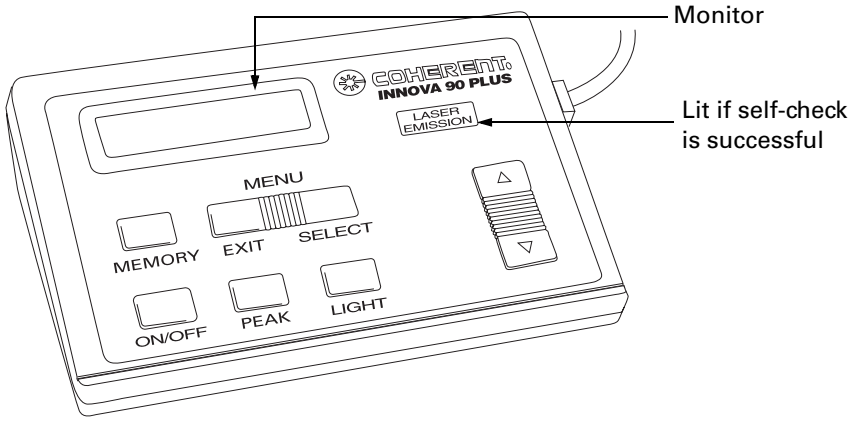
Starting up the FLIPR System

About Start-Up

In order to ensure proper communication between the FLIPR system control software and hardware, the start-up and shut-down procedures described in this chapter should be followed precisely.

Procedure for Start-Up

To startup the FLIPR system:

Step	Action
1	Turn on the laser cooling system.
2	Power-up the laser by first turning the power supply key to the **STANDBY** position.
3	Press the **ON/OFF** switch, located on the laser's remote control (see the figure below). The remote control monitor will display a countdown while the laser performs a self-check. If the self-check is successful, the emission light at the upper right corner of the remote control will remain lit following the completion of the countdown (and the amperage-to-wattage correlation will be shown). The laser typically requires approximately 30 min to warm up and to stabilize. Experiments should not be started until this time has elapsed. **Note** It is not necessary for the laser to stabilize before proceeding with the following start-up steps. 
4	Turn on the computer and the monitor.
5	Simultaneously press the **CTRL/ALT/DEL** keys to launch the Windows NT® operating system. At the prompt, enter your password. **Note** During installation, the computer password is **FLIPR.** **CAUTION Wait for the computer to completely finish booting up before proceeding.**
6	Turn on (or open) the air source and set the air pressure to at least 80 psi.
7	If starting a FLIPR^2, turn on the camera controller. The CCD camera typically reaches its cooled temperature in approximately 5–10 minutes. When the proper temperature is reached, the software's FLIPR status bar displays the correct camera temperature (FLIPR systems using the CST 133 camera controller). For the FLIPR^3 systems, the FLIPR status bar displays **locked**.

To startup the FLIPR system: *(continued)*

Step	Action
8	Turn on the FLIPR system. The power switch is located at the rear of the tower.
9	If starting a FLIPR^2, turn on the vacuum and peristaltic pumps, if using the tip washer.
10	Launch the software by clicking on the FLIPR icon located on the desktop. The pipettor will immediately "home" itself. **Note** Launching the software may take several seconds. Do not repeatedly double-click the software icon.

Shutting Down the FLIPR System

Shut-down Procedure

To shut down the FLIPR system:

Step	Action
1	At the end of a programmed experiment, wait for any pipettor operation to complete and the door to open.
2	Use the laser remote control to turn the laser off, and then turn the laser power supply key to the **OFF** position. **CAUTION To prevent damage to the laser, wait a minimum of 5 minutes after shutting off the laser remote before turning off the cooling system.**
3	Exit the FLIPR software by choosing **Exit** from the **File** menu.
4	Turn off the camera controller, the computer and monitor.
5	Turn off the FLIPR system power switch.
6	If shutting down a FLIPR^2, turn off the vacuum and peristaltic pumps.
7	Turn off (or close) the air source.
8	After a minimum of 5 min, turn off the laser's cooling system.

Software Installation and Description

4

Introduction

Chapter Contents

This chapter provides a description of the windows, menus, dialog boxes, and toolbar icons of the FLIPR® software.

Installing FLIPR v. 2.1.1 Software for Desktop Computers

About Installation for Desktop Computers

These instructions may be used for installation of FLIPR version 2.1.1 software on desktop computers. It may also be used to open FLIPR1 and FLIPR384 data files on a desktop computer.

Note It is possible to install software version 1.26 or below and software version 2.1.1 on the same computer concurrently.

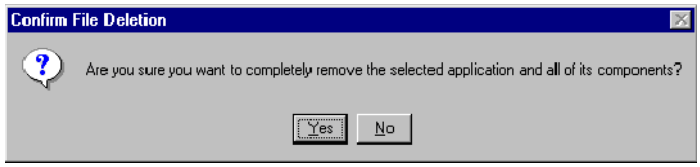
Note To install FLIPR 2.1.1 software, you will need to have administrator privileges on your computer.

IMPORTANT It is not possible to install software versions of 2.X.X on the same computer. Before installing FLIPR 2.1.1 software, previous versions of FLIPR 2.X.X. software must be uninstalled.

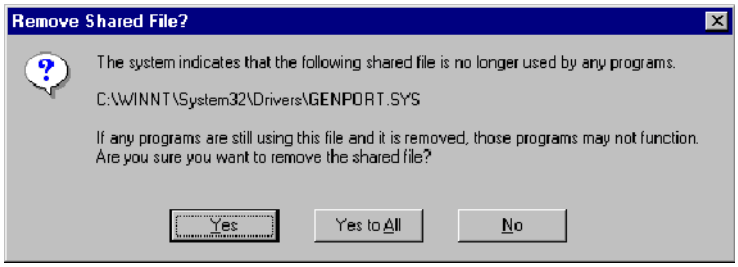
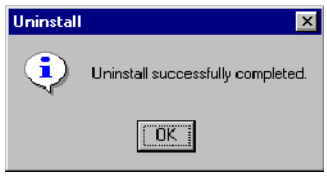
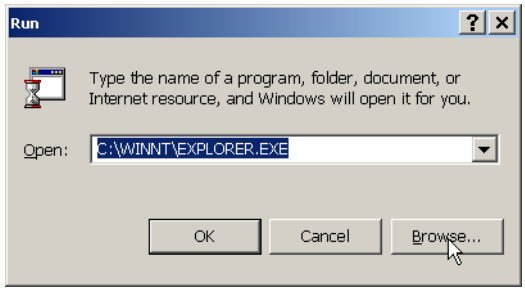
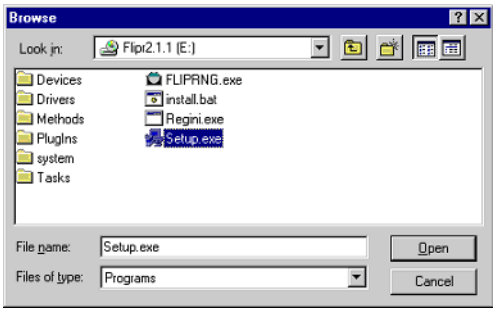
IMPORTANT To install FLIPR version 2.1.1, it is not necessary to uninstall FLIPR version 1.26 or below; however, when installing FLIPR version 2.1.1, make sure that the selected installation directory is different from FLIPR version 1.26 or below.

Installing FLIPR v. 2.1.1 Software on a Desktop Computer

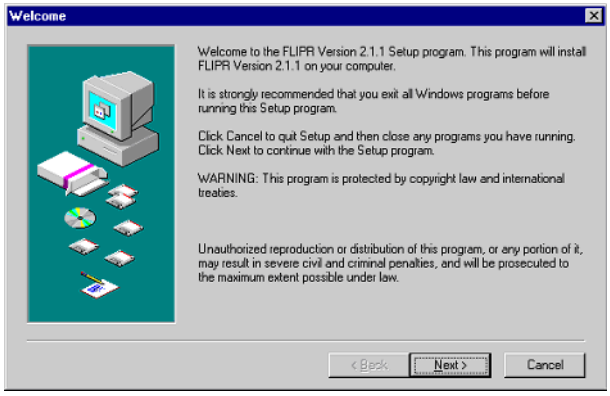
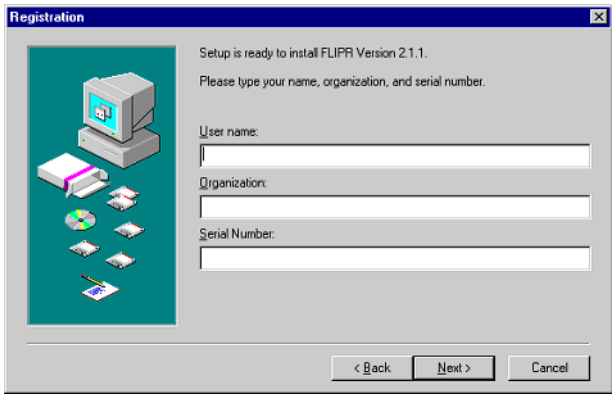
To install the software on a desktop computer:

Step	Action
1	Make sure that all applications are closed.
2	Remove all previous versions of FLIPR v. 2.X.X before installing the new version. a. Click **Start** and select **Programs** > **FLIPR Version XX** > **Remove FLIPR Version XX**. The following dialog box appears.  b. Select **Yes**.

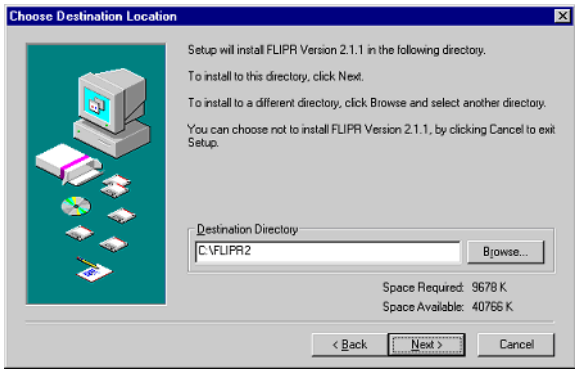
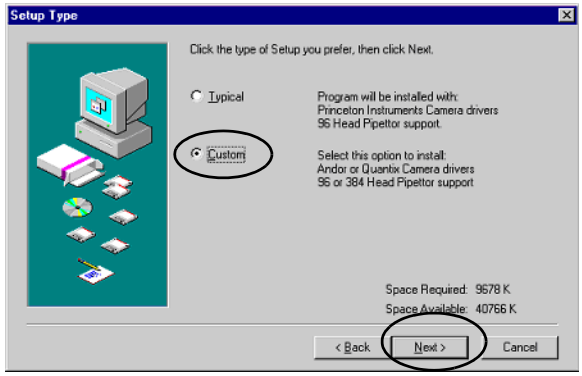
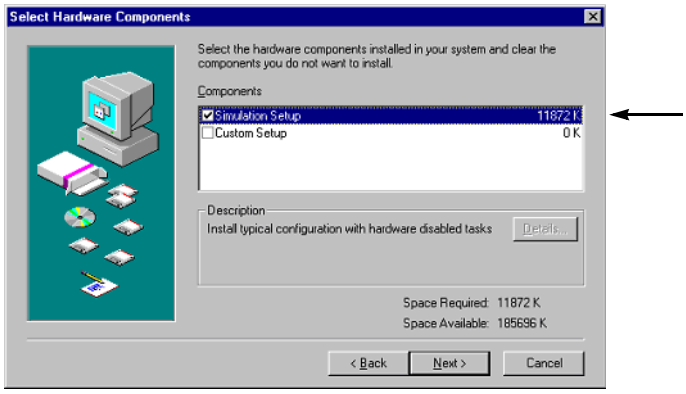
To install the software on a desktop computer: *(continued)*

Step	Action
3	If the **Remove Shared File** dialog box appears, select **NO**.  After the software is removed, the following dialog box will appear. **Note** The removal process will remove only those files and folders installed during the original installation. Data files will not be removed. 
4	Confirm that the installation directory (**C:\Flipr2)** is still present and that the subdirectory **Tasks** has either been deleted or is empty.
5	Insert the FLIPR v. 2.1.1 CD into the CD drive, click **Start** on the operating menu and select **Run**. 
6	Click **Browse**, navigate to the CD drive and select **Setup.exe**. Click **Open** to return to the **Run** dialog box and click **OK** to run the setup program. 

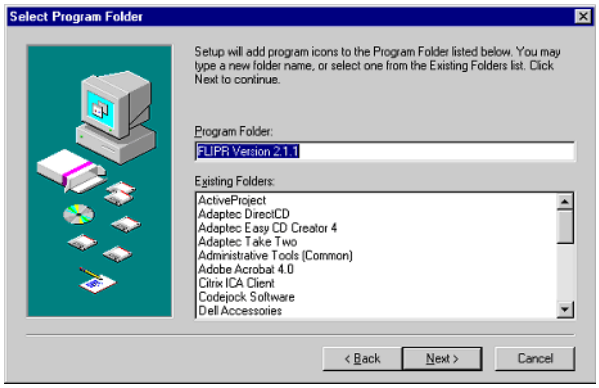
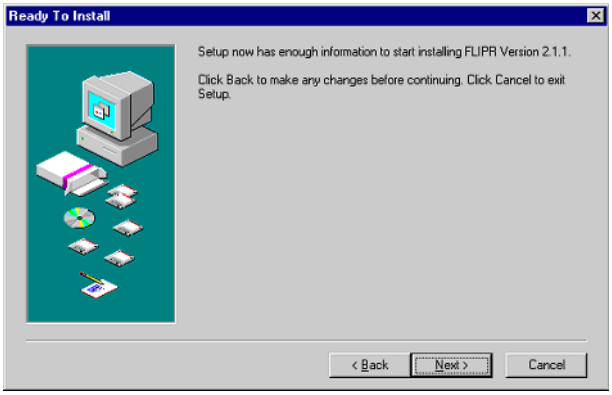
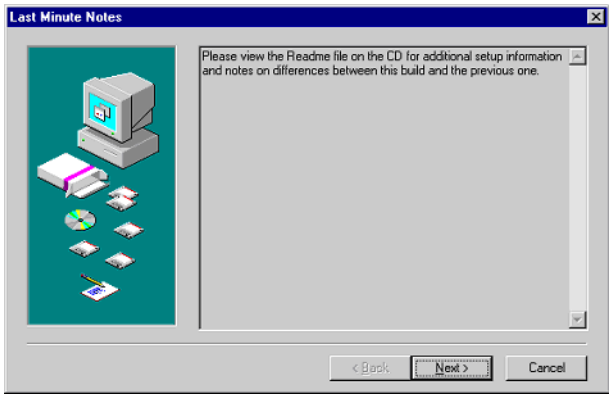
To install the software on a desktop computer: *(continued)*

Step	Action
7	The setup program will display a welcome screen. Click **Next** to continue. 
8	The **Registration** dialog box appears. Enter your **User name** and **Organization** in the appropriate text fields. **Note** No **Serial Number** is required. Click **Next**. 

To install the software on a desktop computer: *(continued)*

Step	Action
9	The destination folder is shown in **Destination DIrectory** field. The default is **C:\FLIPR2**. Select the drive with the largest amount of free space available and enter **drive letter:\FLIPR2**. Click **Next** to continue. 
10	In the **Setup Type** dialog box, select the **Custom** option and click **Next**. 
11	In the **Select Hardware Components** dialog box, uncheck the **Custom Setup** option in the **Components** area and check **Simulation Setup**. Click **Next**. 

To install the software on a desktop computer: *(continued)*

Step	Action
12	In the **Select Program Folder** dialog box, click **Next** to continue. 
13	In the **Ready To Install** dialog box, click **Next** to continue. 
14	After installation, the **Last Minute Notes** dialog box appears. Click **Next**. 

To install the software on a desktop computer: *(continued)*

Step	Action
15	The **Finished** dialog box will appear and will indicate the computer must be restarted. Click **Close** to complete the installation and allow the computer to restart. 

Modifying Simulation Mode From 384- to 96-Well Head

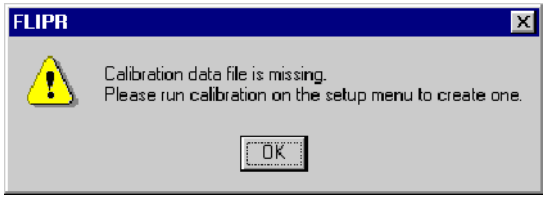
By default, the software will be installed and configured to simulate a system with a 384-well head.

To modify installation to simulate a 96-well head:

Step	Action
1	Close FLIPR software, if it is running.
2	Find the installation directory. By default this will be **C:\Flipr2**.
3	In the installation directory, locate the subdirectory **Tasks**. By default this will be **C:\Flipr2\Tasks**.
4	In the subdirectory **Tasks**, there are two files named **TaskOrder - 96.flp** and **TaskOrder.flp**. Rename the file **TaskOrder.flp** to be **TaskOrder - 384.flp**. Rename the file **TaskOrder - 96.flp** to be **TaskOrder.flp**.
5	Locate the file **SetupNG.flp** in the subdirectory **Tasks**.
6	Open the **SetupNG.flp** file in a text editor.
7	Set **PIPETTORTYPE=96**.
8	Set **HARDWARE=0**.
9	Save and close the file.
10	Start the FLIPR software.

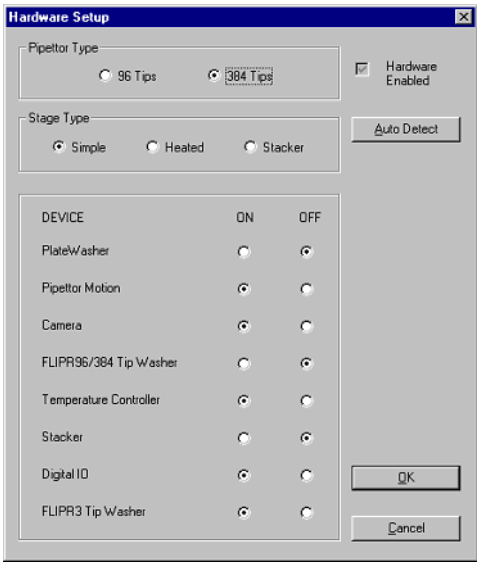
Running the Software in Simulation Mode

In simulation mode you will not be able to create a calibration file. Ignore the following message when it appears.



Note When the simulation software starts, all the hardware is set to OFF. In this state you will be unable to set up an experiment as most of the tasks are not available. To be able to set up an experiment which could be transferred onto a FLIPR system, you must configure the software to match the system on which you will be performing the experiment.

To modify experimental protocols with a simulation installation:

Step	Action
1	Start the FLIPR software.
2	From the main menu, select **Hardware** from the **Setup** menu. This opens the **Hardware Setup** dialog box. 
3	Do not click **Auto Detect**. This will provide inaccurate information in the simulation mode.
4	If the head-type displayed does not match the type that will be used on the system simulated, see *"Modifying Simulation Mode From 384- to 96-Well Head"* on page 4-7 for instructions.
5	Select the **Stage** to match the system that you will be using.
6	Turn on those **Devices** to match the instrument that you will be using.

To modify experimental protocols with a simulation installation: *(continued)*

Step	Action
7	The following **Devices** must always be turned ON: ♦ Pipettor Motion ♦ Camera ♦ Temperature Controller ♦ Digital IO
8	If simulating a system with a **Stacker**, turn on the **Stacker** device and select the **Stacker** stage-type.
9	If simulating a FLIPR^{96} or FLIPR^{384} system with a tipwasher, turn on the appropriate tipwasher for this instrument.
10	Click **OK**.

Installing FLIPR v. 2.1.1 Software; Upgrading from v. 1.26

About Upgrading to Software v. 2.1.1 from v.1.26

These instructions can be used to install FLIPR version 2.1.1 software on FLIPR^{96}, FLIPR^{384}, FLIPR^{2}, and FLIPR^{3} instruments. This software does not support FLIPR1 instruments.

It is possible to install FLIPR version 1.26 or below and FLIPR version 2.1.1 on the same instruments concurrently.

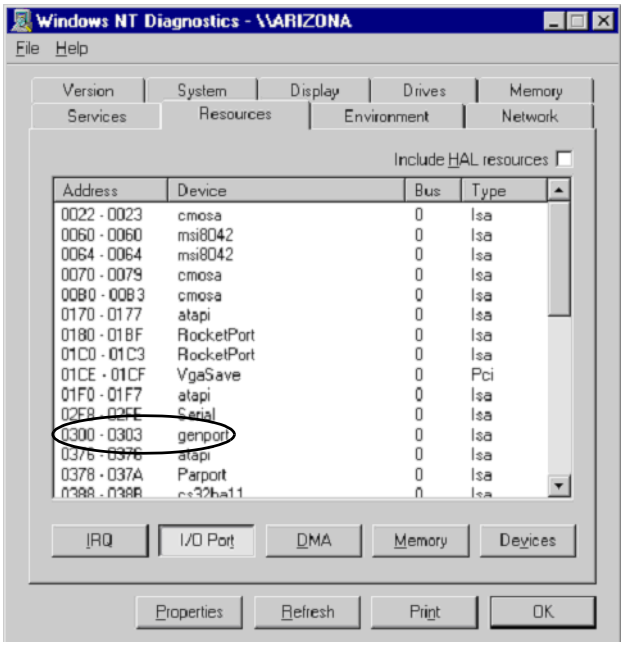
IMPORTANT It is NOT possible to install multiple versions of FLIPR 2.X.X on the same instrument. Before installing a new version of FLIPR 2.1.1, previous versions of FLIPR 2.X.X software must be uninstalled.

Tasks to do Before Installation

- Make sure that you have administrator privileges on the instrument computer.
- Determine the camera type and the pipettor head type
  - The camera will be either a Princeton Instruments (FLIPR^{96}, FLIPR^{384}, FLIPR^{2}) or an Andor (FLIPR^{3}).
  - The pipettor head will be either a 96- or 384-well plate type.
- Make a backup copy of the **Setup.flp** file from the old version of FLIPR software on a floppy disk prior to starting the installation. This file can be found in the installation directory (**C:\Flipr**) inside the subdirectory bin.
- Record the address for the digital I/O card

Recording Digital I/O Address

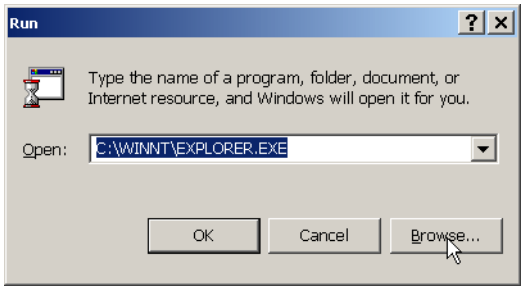
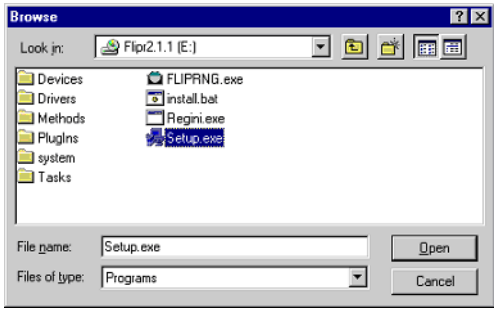
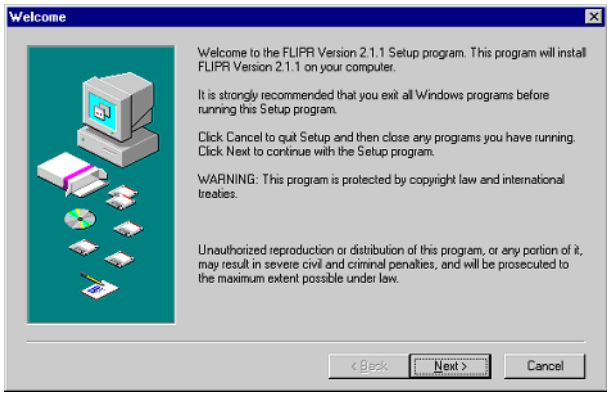
To determine the digital I/O address:

Step	Action
1	Click **Start** and select **Programs** > **Administrative Tools** (common) > **Windows NT Diagnostics**.
2	In the **Windows NT Diagnostics** dialog box, select the **Resources** tab Click **I/O Port** 
3	There should be a device named **genport**. Note the address for this device. In the example below, the address is 0300-0303. You need only the first part of this address (in the example 0300). Record the number here _____.
4	Close the **Windows NT Diagnostics** dialog box.
5	Click **Start** and select **Programs** > **Accessories** > **Calculator**.
6	In the **Calculator** dialog box, select **Scientific** from the **View** menu.
7	Select **Hex**.
8	Enter the recorded number in the text field.
9	Select **Dec** to convert the entered hexadecimal address to decimal. Record the address here _____.

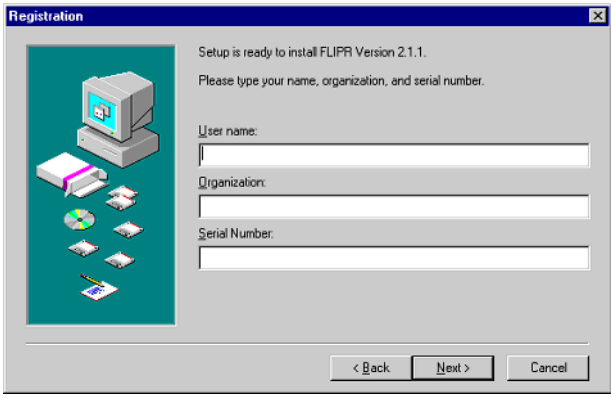
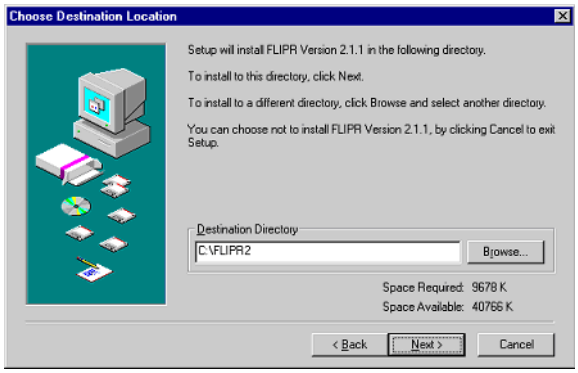
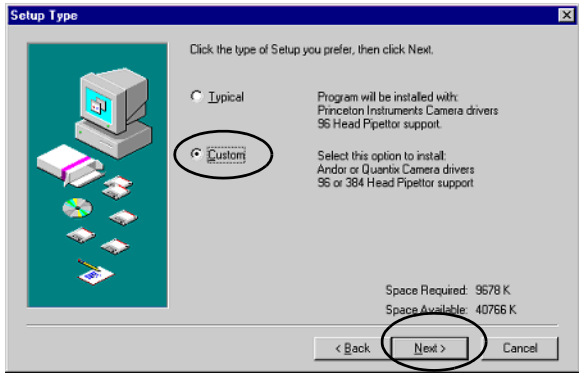
Installing FLIPR Software v. 2.1.1 on Instruments Running v. 1.26

Make sure that all applications running on the computer are closed.

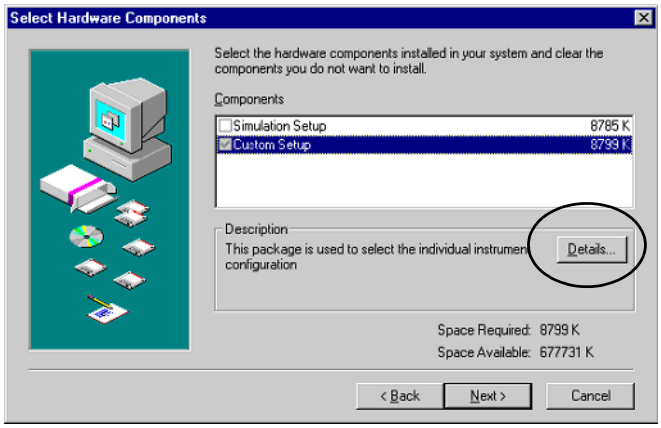
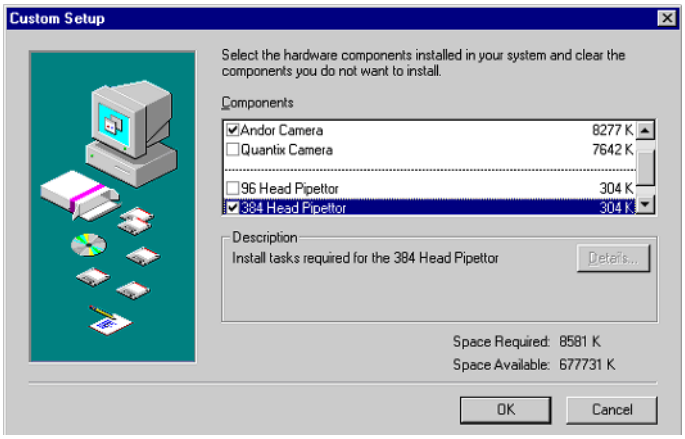
To upgrade to FLIPR software 2.1.1 from v. 1.26 or below:

Step	Action
1	Insert the FLIPR v. 2.1.1 CD into the CD drive, click **Start** on the operating menu and select **Run**. 
2	Click **Browse**, navigate to the CD drive and select **Setup.exe**. Click **Open** to return to the **Run** dialog box and click **OK** to run the setup program. 
3	The setup program will display a welcome screen. Click **Next** to continue. 

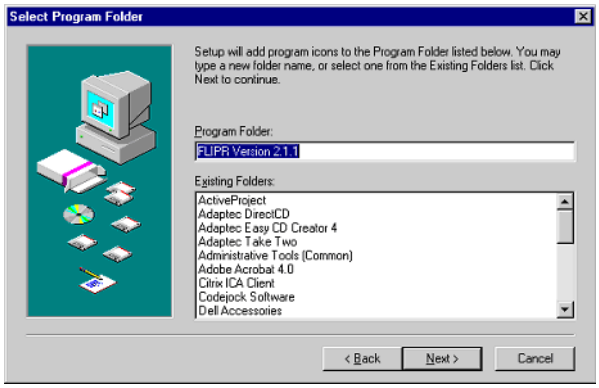
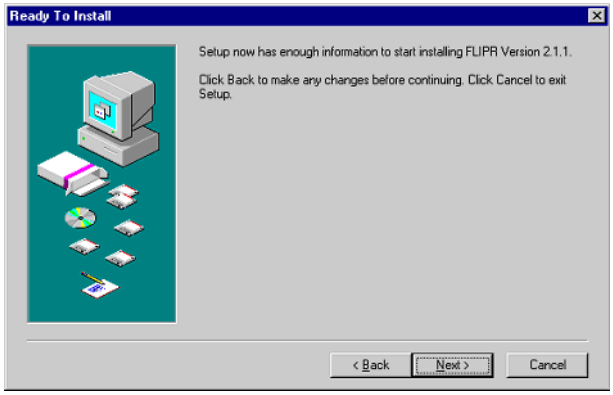
To upgrade to FLIPR software 2.1.1 from v. 1.26 or below: *(continued)*

Step	Action
4	The **Registration** dialog box appears. Enter your **User name** and **Organization** in the appropriate text fields. **Note** No **Serial Number** is required. Click **Next**. 
5	The destination folder is shown in **Destination DIrectory** field. The default is **C:\Flipr2**. Select the drive with the largest amount of free space available and enter **drive letter:\Flipr2**. Click **Next** to continue. 
6	In the **Setup Type** dialog box, select the **Custom** option and click **Next**. 

To upgrade to FLIPR software 2.1.1 from v. 1.26 or below: *(continued)*

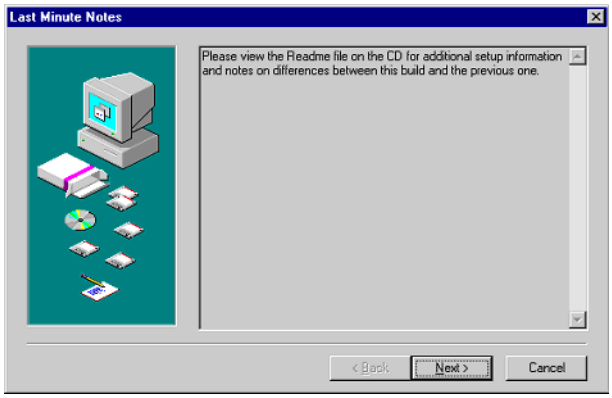
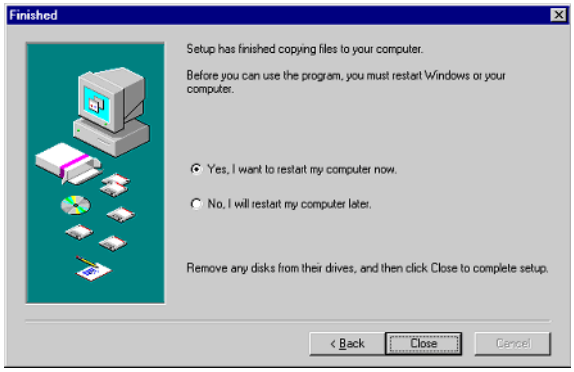
Step	Action
7	In the **Select Hardware Components** dialog box, check **Custom Setup** and uncheck **Simulation Setup**. Click **Details**. 
8	In the **Custom Setup** dialog box, select the appropriate camera (either Princeton Instruments or Andor) and pipettor head (either 96- or 384-head). **IMPORTANT** Make sure that all other options are unchecked to prevent incorrect software components from being installed. Click **OK** to continue. **Note** The picture below is only an example. Check the appropriate camera type and pipettor head in the **Components** field. 
9	In the **Select Hardware Components** dialog box, click **Next** to continue.

To upgrade to FLIPR software 2.1.1 from v. 1.26 or below: *(continued)*

Step	Action
10	In the **Select Program Folder** dialog box, click **Next** to continue. 
11	In the **Ready To Install** dialog box, click **Next** to continue. 
12	The installer requires information about the digital I/O card installed in the system. The **Digital I/O Setup** dialog box will appear asking if the I/O card is a PCI-type card. Supply the digital I/O card information: a. Check the front of the PC to find the system model name and number. refer to the table below to determine the I/O card type.

Computer System Description	Digital I/O Card Type	Hex Address	Decimal Address
Dell Dimension XPS w/JAZ drive	ISA	N/A	N/A
Dell Optiplex GX1 w/JAZ drive	ISA	N/A	N/A
Dell Optiplex GX110 w/JAZ drive	PCI	e8bc	59580
Dell Optiplex GX110 w/CD-RW	PCI	Ecbc	60604
Dell Optiplex GX150 w/CD-RW	PCI	ec78	60536

To upgrade to FLIPR software 2.1.1 from v. 1.26 or below: *(continued)*

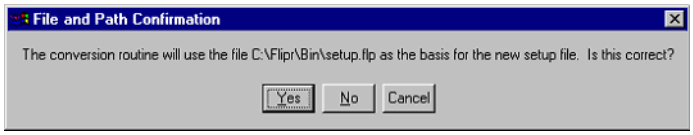
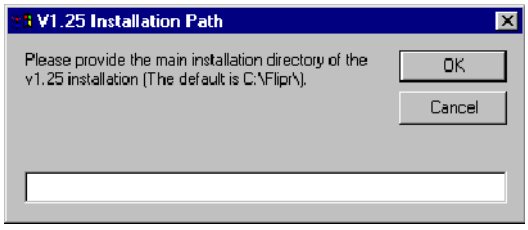
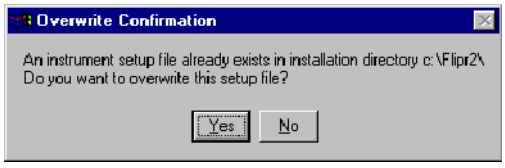
Step	Action
13	Click **Yes** to indicate a PCI-type card or **No** to indicate an ISA-type card.
14	A base address is required for the PCI-type cards. When prompted, use the decimal address that you calculated in step 1. If you failed to obtain this address, use the default value listed above. **Note** The installer may determine the address of a PCI-type card automatically. If the installer is unable to locate a record indicating the base address, it will request one. The installer requires the correct DECIMAL base address. You should have determined the correct address in step 1 of this procedure. **Note** The ISA-type digital I/O card does not require a specific address.
15	After installation, the **Last Minute Notes** dialog box appears. Click **Next**. 
16	The **Finished** dialog box will appear and will indicate the computer must be restarted. Click **Close** to complete the installation and allow the computer to restart. 

Creating a New Setup File

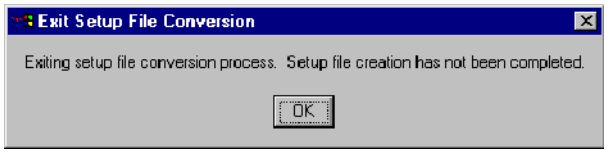
After installation, it will be necessary to create a setup (a.k.a., configuration) file for the FLIPR instrument. When FLIPR software was originally installed, this file was created by MDC personnel.

This version of the software has a special routine that allows you to create a new setup file from the setup file created for v. 1.26 and earlier.

To create a new configuration file:

Step	Action
1	Close the FLIPR software if it is running.
2	Locate the installation directory. By default this is **C:\Flipr2**.
3	Within the installation directory, locate the subdirectory **System**. By default this is **C:\Flipr2\System**.
4	In the subdirectory **System**, locate the file **SampleSetupNG.flp** and rename it **SetupNG.flp**.
5	Open the renamed file in a text editor. Confirm there is a line that reads: **HARDWARE=1** (//0 is for no hardware, 1 is for hardware)
6	Close the text editor and start the FLIPR software. **Note** The FLIPR software will attempt to access hardware while starting the software. There may be some error messages during this process since the instrument is not yet correctly configured.
7	Open any data file.
8	Select **Convert v 1.26 Setup Files** from **Special Operations** located under the **Run** menu.
9	The FLIPR software will attempt to locate the setup file for the previous FLIPR software version. If it is able to find the desired file, it will display the following dialog. Click **OK** if the indicated path is correct. 
10	If the FLIPR software is unable to identify the previous installation path, the following dialog will appear. Enter the correct path. 
11	When the following warning appears, click **Yes**. 

To create a new configuration file: *(continued)*

Step	Action
12	The software will proceed to create the required new setup file. When it is complete, the following message is displayed. Click **OK** to finish the setup file creation.  **Note** If any errors occur during the conversion of a setup file, the following message will appear. This message indicates that a new setup file was not created. One must be created to run the FLIPR system with the new software. If this occurs, contact Molecular Devices Technical Support at 800-635-5577 ext. 1. 
13	Exit the software.
14	Locate the installation directory. By default this is **C:\Flipr2**.
15	Locate the subdirectory **System**.By default this is **C:\Flipr2|System**.
16	Delete the file named **SetupNG.flp.**
17	From the floppy disk that has the saved **Setup.flp** file, copy it to the **System** subdirectory and rename it **SetupNG.flp**.
18	Restart the FLIPR software. The software should now use the new setup file.

Installing FLIPR Software v. 2.1.1; Upgrading from v. 2.0

About Upgrading to Software v. 2.1.1 from v. 2.0

These instructions can be used to install FLIPR version 2.1.1 software on FLIPR^{96}, FLIPR^{384}, FLIPR^{2}, and FLIPR^{3} instruments. This software does not support FLIPR1 instruments.

It is possible to install FLIPR version 1.26 or below and FLIPR version 2.1.1 on the same instruments concurrently.

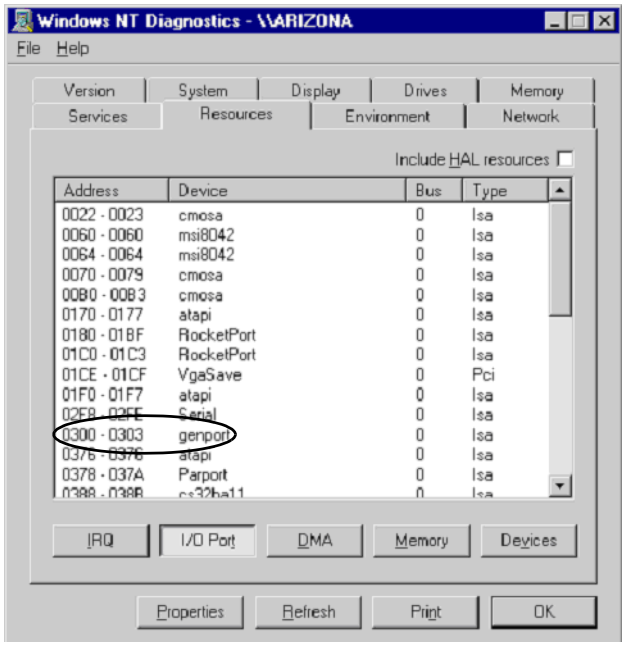
IMPORTANT It is NOT possible to install multiple versions of FLIPR 2.X.X on the same instrument. Before installing a new version of FLIPR 2.1.1, previous versions of FLIPR 2.X.X software must be uninstalled.

Tasks to do Before Installation

- Make sure that you have administrator privileges on the instrument computer.
- Determine the camera type and the pipettor head type
  - The camera will be either a Princeton Instruments (FLIPR^{96}, FLIPR^{384}, FLIPR^{2}) or an Andor (FLIPR^{3})
  - The pipettor head will be either a 96- or 384-well plate type.
- Make a copy of files unique to the instrument. The files are found in the installation subdirectory **System** (by default this is **C:\Flipr2\System**. These files are:
  - Calibration.dat
  - PlateLibrary.dat
  - SetupNG.flp
- Make a backup copy of the **Setup.flp** or **SetupNG.flp** file from the old version of FLIPR software on a floppy disk prior to starting the installation. This file can be found in the installation directory (**C:\Flipr**) inside the subdirectory bin.
- Check address for the digital I/O card
- Delete older versions of FLIPR software

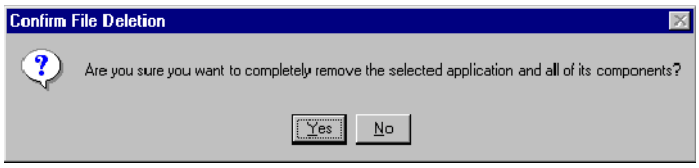
Recording Digital I/O Address

To determine the digital I/O address:

Step	Action
1	Click **Start** and select **Programs** > **Administrative Tools** (common) > **Windows NT Diagnostics**.
2	In the **Windows NT Diagnostics** dialog box, select the **Resources** tab Click **I/O Port** 
3	There should be a device named **genport**. Note the address for this device. In the example below, the address is 0300-0303. You need only the first part of this address (in the example 0300). Record the number here _____.
4	Close the **Windows NT Diagnostics** dialog box.
5	Click **Start** and select **Programs** > **Accessories** > **Calculator**.
6	In the **Calculator** dialog box, select **Scientific** from the **View** menu.
7	Select **Hex**.
8	Enter the recorded number in the text field.
9	Select **Dec** to convert the entered hexadecimal address to decimal. Record the address here _____.

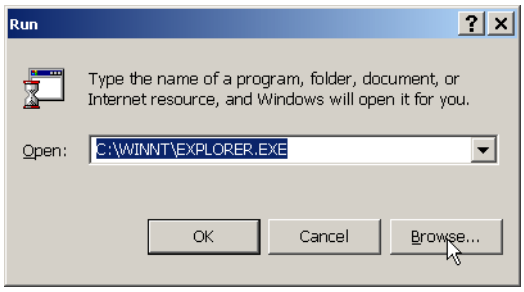
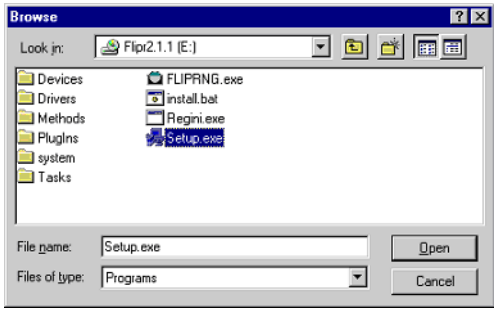
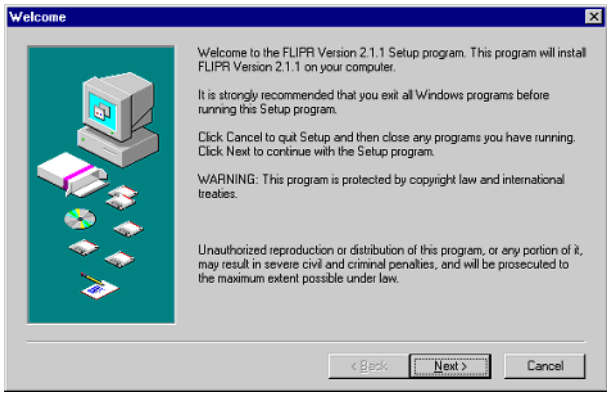
Removing Previous Software Versions

To remove older versions of FLIPR software:

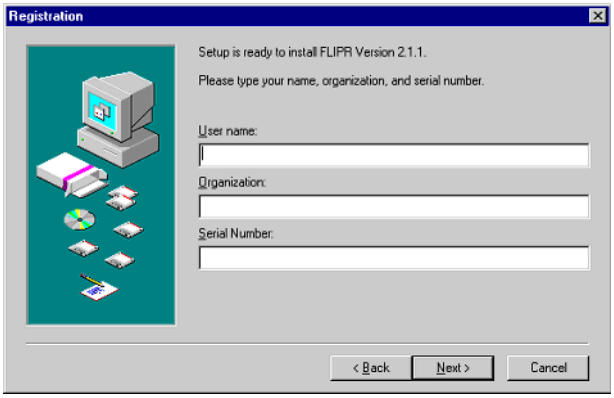
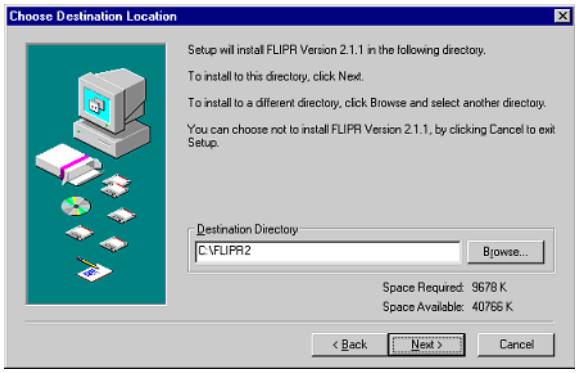
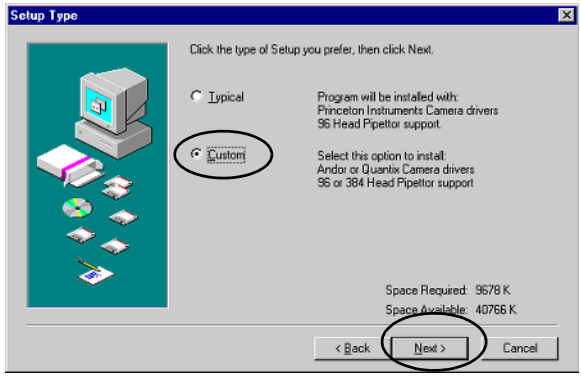
Step	Action
1	Remove all previous versions of FLIPR v. 2.X.X before installing the new version. a. Click **Start** and select **Programs** > **FLIPR Version XX** > **Remove FLIPR Version XX**. The following dialog box appears.  b. Select **Yes**.
2	If the **Remove Shared File** dialog box appears, select **NO**.  After the software is removed, the following dialog box will appear. **Note** The removal process will remove only those files and folders installed during the original installation. No new files or data files will be removed or modified. The custom configuration file for this instrument (**SetupNG.flp**) will NOT be removed. 
3	Confirm that the installation directory (**C:\Flipr2)** is still present and that the subdirectory **Tasks** has either been deleted or is empty.

Installing FLIPR Software v. 2.1.1 on Instruments Running v. 2.0

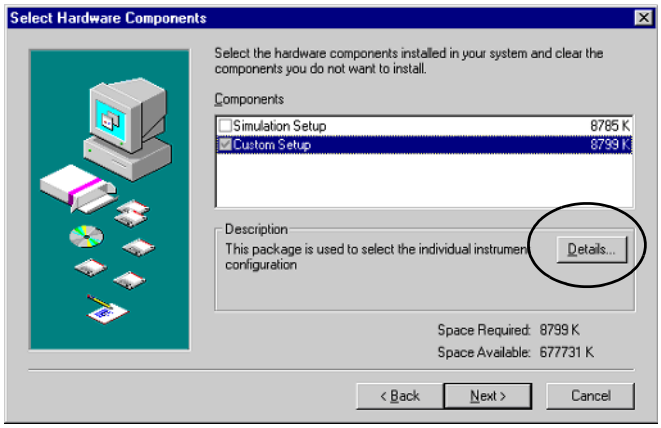
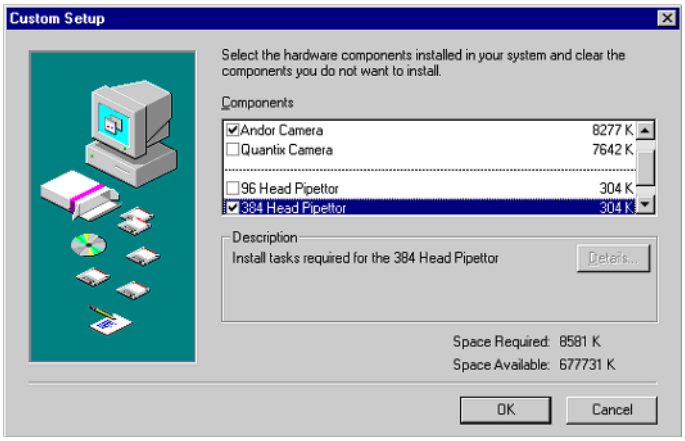
To install software version 2.1.1 on instruments that have version 2.0:

Step	Action
1	Close all applications that are running, insert the FLIPR v. 2.1.1 CD into the CD drive, click **Start** on the operating menu and select **Run**. 
2	Click **Browse**, navigate to the CD drive and select **Setup.exe**. Click **Open** to return to the **Run** dialog box and click **OK** to run the setup program. 
3	The setup program will display a welcome screen. Click **Next** to continue. 

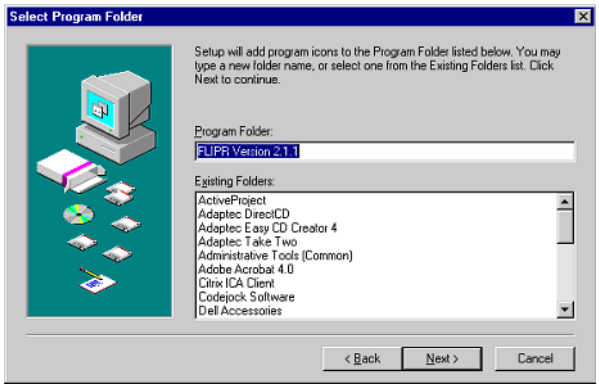
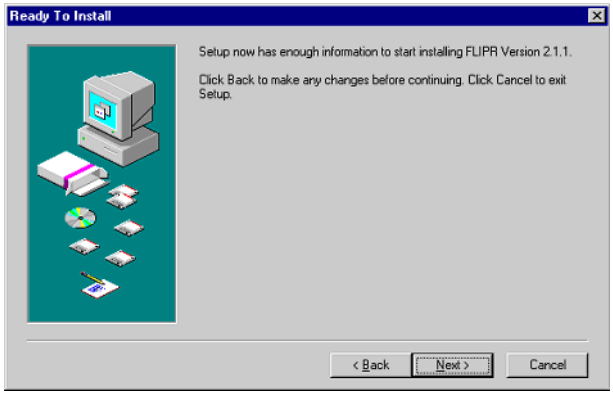
To install software version 2.1.1 on instruments that have version 2.0: *(continued)*

Step	Action
4	The **Registration** dialog box appears. Enter your **User name** and **Organization** in the appropriate text fields. **Note** No **Serial Number** is required. Click **Next**. 
5	The destination folder is shown in **Destination DIrectory** field. The default is **C:\Flipr2**. Select the drive with the largest amount of free space available and enter **drive letter:\Flipr2**. Click **Next** to continue. 
6	In the **Setup Type** dialog box, select the **Custom** option and click **Next**. 

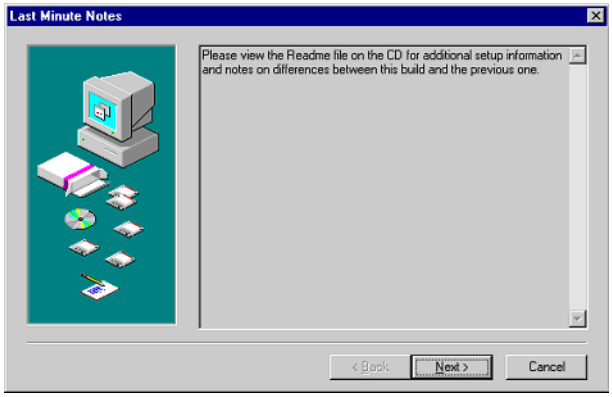
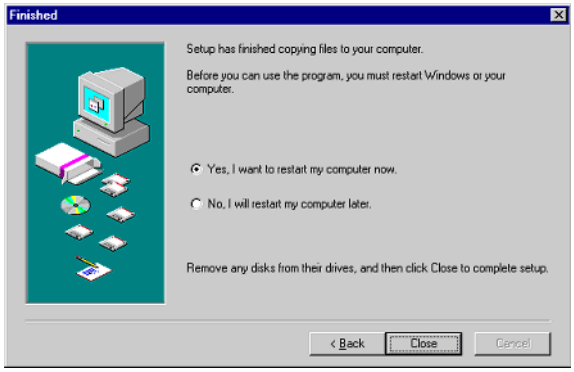
To install software version 2.1.1 on instruments that have version 2.0: *(continued)*

Step	Action
7	In the **Select Hardware Components** dialog box, check **Custom Setup** and uncheck **Simulation Setup**. Click **Details**. 
8	In the **Custom Setup** dialog box, select the appropriate camera (either Princeton Instruments or Andor) and pipettor head (either 96- or 384-head). **IMPORTANT** Make sure that all other options are unchecked to prevent incorrect software components from being installed. Click **OK** to continue. **Note** The picture below is only an example. Check the appropriate camera type and pipettor head in the **Components** field. 
9	In the **Select Hardware Components** dialog box, click **Next** to continue.

To install software version 2.1.1 on instruments that have version 2.0: *(continued)*

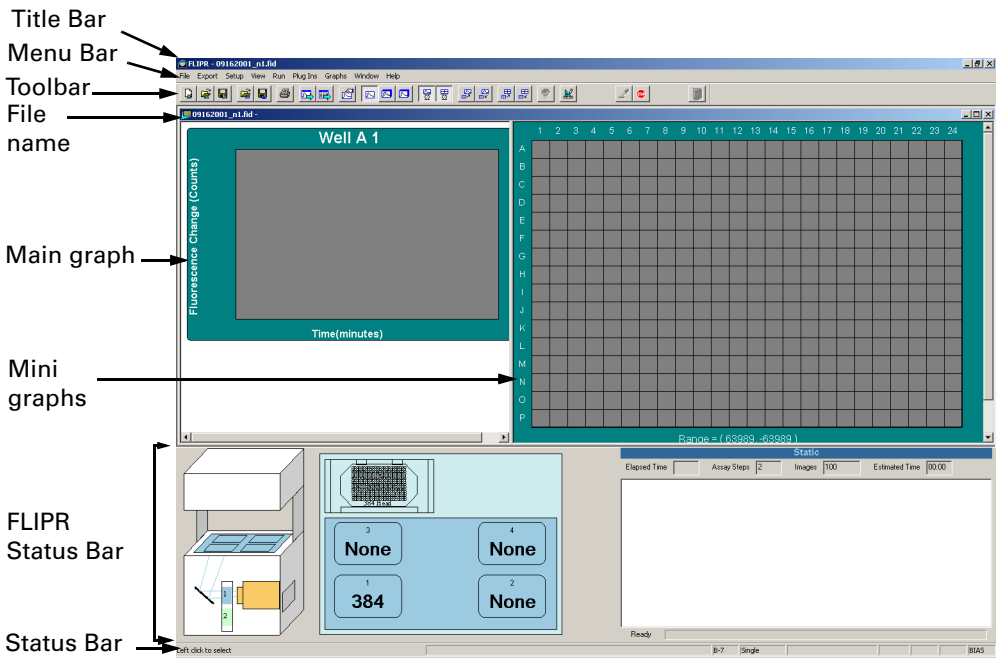
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>10</td><td>In the Select Program Folder dialog box, click Next to continue.
 
</td></tr>
<tr><td>11</td><td>In the Ready To Install dialog box, click Next to continue.
 
</td></tr>
<tr><td>12</td><td>The installer requires information about the digital I/O card installed in the system. The Digital I/O Setup dialog box will appear asking if the I/O card is a PCI-type card.
Supply the digital I/O card information:
a. Check the front of the PC to find the system model name and number. Refer to the table below to determine the I/O card type.
<table>
<tr><th>Computer System Description</th><th>Digital I/O Card Type</th><th>Hex Address</th><th>Decimal Address</th></tr>
<tr><td>Dell Dimension XPS w/JAZ drive</td><td>ISA</td><td>N/A</td><td>N/A</td></tr>
<tr><td>Dell Optiplex GX1 w/JAZ drive</td><td>ISA</td><td>N/A</td><td>N/A</td></tr>
<tr><td>Dell Optiplex GX110 w/JAZ drive</td><td>PCI</td><td>e8bc</td><td>59580</td></tr>
<tr><td>Dell Optiplex GX110 w/CD-RW</td><td>PCI</td><td>Ecbc</td><td>60604</td></tr>
<tr><td>Dell Optiplex GX150 w/CD-RW</td><td>PCI</td><td>ec78</td><td>60536</td></tr>
</table></td></tr>
</table>

To install software version 2.1.1 on instruments that have version 2.0: *(continued)*

Step	Action
13	Click **Yes** to indicate a PCI-type card or **No** to indicate an ISA-type card.
14	A base address is required for the PCI-type cards. When prompted, use the decimal address that you calculated in *"Recording Digital I/O Address"* on page 4-20. If you failed to obtain this address, use the default value listed above. **Note** The installer may determine the address of a PCI-type card automatically. If the installer is unable to locate a record indicating the base address, it will request one. The installer requires the correct DECIMAL base address. You should have determined the correct address in step 1 of this procedure. **Note** The ISA-type digital I/O card does not require a specific address.
15	After installation, the **Last Minute Notes** dialog box appears. Click **Next**. 
16	The **Finished** dialog box will appear and will indicate the computer must be restarted. Click **Close** to complete the installation and allow the computer to restart. 
17	After completing the software installation, copy the saved files from *"Tasks to do Before Installation"* on page 4-19 to the installation subdirectory **System**.

Main Window

The FLIPR Software Main Window



The main window of the FLIPR software includes a title bar, menu bar, tool bar, file name, main and mini graphs, FLIPR status bar, and status bar.

Note The default view for the software is a window that fills the screen. You can size this window to be smaller.

Title Bar

The title bar extends across the top of the window, inside the window borders. It displays the name of the application followed by the name of the assay or data file. The color of the title bar indicates whether or not it is the active window: 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 may be dimmed).

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

FLIPR Software Menus

Menu Bar

The menu bar is the area across the top of the window directly below the title bar. It contains the names of the menus that group together related commands. The **File** and **Export** menus control the export and print functions, as well as management of the control and data files. The **Setup** menu allows you to define all the experimental parameters. The **Run** menu controls the FLIPR system's functions such as beginning an experiment and taking a plate view. The **Graph**, **Window**, and **View** menus control the display of the data. The **Plug Ins** menu provides access to the diagnostic, software development, and optional functions.

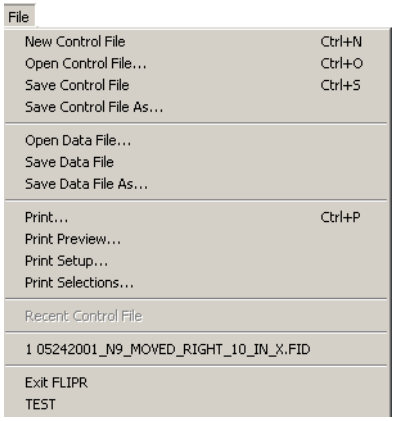
Clicking on a menu name displays a list of commands that can be used to access software functions. The contents of these menus are described below.

Note The contents of each menu will vary depending on whether or not a control or data file is open. The descriptions provided here are from menu items present when a control file is open.

File Menu

The top portion of the **File** menu contains items that affect FLIPR control files (*.fcf). These are control files containing all of the parameters to run an assay, display graphs, and export data.

Control files are an important feature of the software, because they allow you to rapidly set and save all experimental parameters to known and optimized conditions without having to reopen all of the relevant dialog boxes.

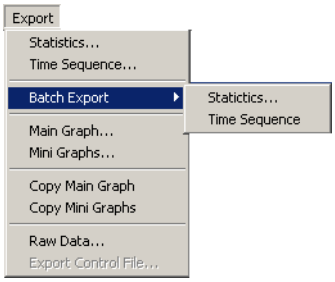


Item	Description
New Control File	Opens a new control file with the default settings.
Open Control File	Opens a previously saved control file.
Save Control File	Saves an unsaved or modified control file.

Item	Description
Save Control File As	Saves a control file under a new name. **Note** The **Save Control File** or **Save Control File As** functions can be used at the end of an assay likely to be repeated. For subsequent runs of the same assay, the saved *.fcf file can be opened and used with all the experimental parameters already set.
Open Data File	Opens a previously saved *.fid or *.fwd data file. You may view multiple data files simultaneously.
Save Data File	Saves the currently active data file. **Note** Data is automatically saved with a predetermined file name at the end of an experimental run. This feature allows you to save the data following additional data manipulations using some of the options found in the **Graphs** menu.
Save Data File As	Saves a data file with a name other than the name automatically assigned by the software.
Print	Opens the **Print** dialog box that enables you to print whatever has been selected in the **Print Selection** dialog box (see *"Print Selections Dialog box"* on page 4-57).
Print Preview	Displays the item to be printed exactly how it will look when it is printed.
Print Setup	Opens the **Print Setup** dialog box that enables you to change or review the printer settings, paper size, and paper orientation.
Print Selection	Opens the **Print Selection** dialog box that enables you to choose the items that will print (main graph, mini graph, and experimental parameters).
Recent Control File	Allows you to open a recent (last four) control file.
1–10 Data Files	Lists the data files that were opened most recently (the most recent file is labeled 1).
Exit	Closes the software program. If you have unsaved data, you will be prompted to save it before closing.

Export Menu

The **Export** menu allows you to save FLIPR data as ASCII text. This feature can be activated automatically by checking the **Export Time Sequence/Statistics after Experiment** box in the **Experiment Setup** dialog box.



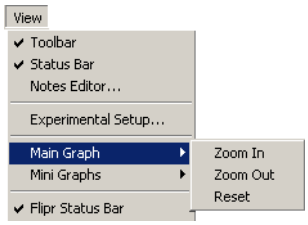
Item	Description
Statistics	Opens the **Export Statistics** dialog box that allows you to define the settings for file naming, data format, and statistics for exporting the statistics data file.
Time Sequence	Opens the **Time Sequence** dialog box that allows you to define settings for file naming and format for exporting the time sequence data file.
Batch Export	For use when you are exporting multiple files into one file. Opens the **Export Statistics** and **Time Sequence** dialog boxes and allows you to modify these settings for a batch of files. **Note** All data files must be closed prior to selecting the batch export function.
Main Graph	Opens the **Export** dialog box and allows you to save the main graph as a FLIPR graph window media file (*.wmf). This file can then be opened for viewing in other types of software.
Mini Graphs	Opens the **Export** dialog box and allows you to save the mini graphs as a FLIPR graph *.wmf file for viewing in other types of software.
Copy Main Graph	Places a copy of the main graph image on the clipboard.
Copy Mini Graphs	Places a copy of the mini graph images on the clipboard.
Raw Data	Exports raw pixel data in *.fid type of file format. **Note** Files are large.
Export Control File	Exports the current control file (*.fcf) to a designated location. **Note** This feature is not currently available.

Setup Menu

Item	Description
Experiment	Opens the **Experiment Setup** dialog box that allows you to program the parameters and conditions of the experiment.
File Directories	Opens the **Default Directories** dialog box that allows you to edit the file names and to choose the location for saving data files.
Plate Library	Opens the **Plate Library** dialog box that is used for adding new plate types to the software.
Calibration	Opens the **Create Calibration Data** window that allows you to create a calibration file. **Note** This option is only available when an instrument is connected to the software.
Heaters	Opens the **Heater Setup** dialog that allows you to set the stage temperature and limits. **Note** This option is only available when the heated stage is connected to the instrument.
Hardware	Opens the **Hardware Setup** dialog box that enables you to designate which hardware devices are available.

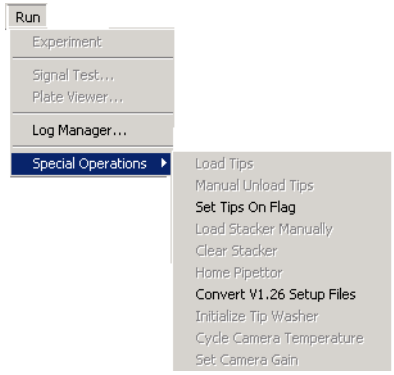
View Menu

Note The **View** menu will not display the **Experimental Setup**, **Main Graph**, or **Mini Graph** screens unless a data file is loaded.



Item	Description
Toolbar	When checked, displays the **Toolbar** (see *"The FLIPR Software Main Window"* on page 4-27).
Status Bar	When checked, displays the **Status Bar** (see *"The FLIPR Software Main Window"* on page 4-27).
Notes Editor	Opens the **Notes Editor** dialog box that allows you to make notes to be saved with a specific data file. The notes will be printed if it is checked in the **Print Selections** dialog box of the **File** menu.
Experimental Setup	Opens the view **Experiment Setup** dialog box that is used to view the parameters and conditions for the data file currently open.
Main Graphs	Controls the viewing magnification of the main graph. Allows you to magnify (**Zoom In**), de-magnify (**Zoom Out**), or return (**Reset**) to its default size.
Mini Graphs	Controls the viewing magnification of the mini graph. Allows you to magnify (**Zoom In**), de-magnify (**Zoom Out**), or return (**Reset**) to its default size.
FLIPR Status Bar	When checked, displays the **FLIPR Status Bar**. It provides a protocol summary and system's current state (see *"The FLIPR Software Main Window"* on page 4-27).

Run Menu



Item	Description
Experiment (Stop)	A toggle that starts or stops the experiment run. **Note** The STOP button should only be used in emergencies. If this button is used, the software should be exited and restarted before running further experiments.

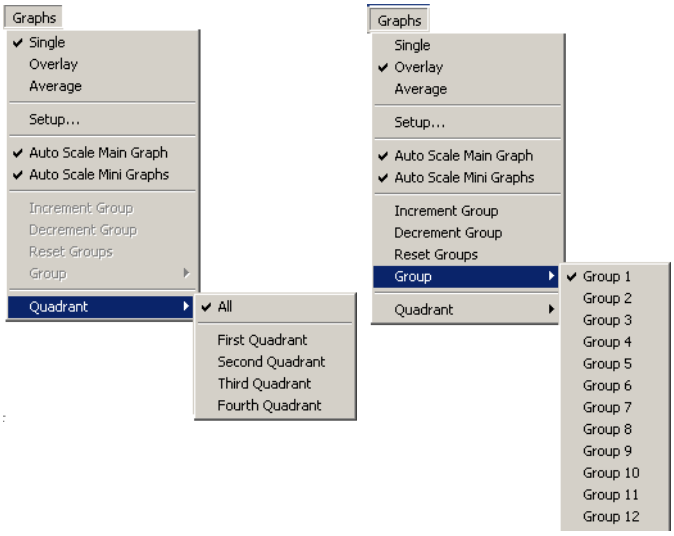
Item	Description
Signal Test	Initiates a signal test of the instrument and then displays the results in the **Signal Test** dialog box. **Note** This feature is only available when connected to the hardware.
FID Viewer	Opens the **FID Viewer** that displays the recorded data from the currently active data file as an image. **Note** In order to view data as images, you must choose to save the data in *.fid format.
Plate Viewer	Opens the **Plate Viewer** dialog box whose function enables the FLIPR system to acquire an image of the current plate using the current settings. **Note** This feature is only available when connected to an instrument.
Log Manager	Opens the **Log Manager** dialog box that displays a list all of the stored log files and allows you to view their contents and to delete them.
Special Operations	This pull-out menu presents different commands that control specific hardware operations. **Note** Some of the following options are inactive if their associated hardware is not available. **Load Tips**—Instructs the pipettor to load tips. **Manual Unload Tips**—Instructs the pipettor to unload tips. **Set Tips on Flag**—Instructs the software that there are tips on the pipettor. This command is typically used when you exit the software while tips are on the pipettor. **Clear Stacker**—Removes plates from the stacker stage and returns them to the stacker. **Home Pipettor**—Resets the pipettor. **Convert v 1.26 Setup Files**—Opens the convert setup files dialog box (see *"Convert Setup Files Dialog Box"* on page 4-73 for more information). **Initialize Tip Washer—**Runs the tip washer setup routine. **Cycle Camera Temperature**—Increases the temperature of the camera to ambient temperature and then decreases it back down to operating temperature. **Set Camera Gain**—Allows you to manually set the camera gain.

Plug Ins Menu

The **Plug Ins** menu contains commands and options that are used to access optional software features and service utilities.

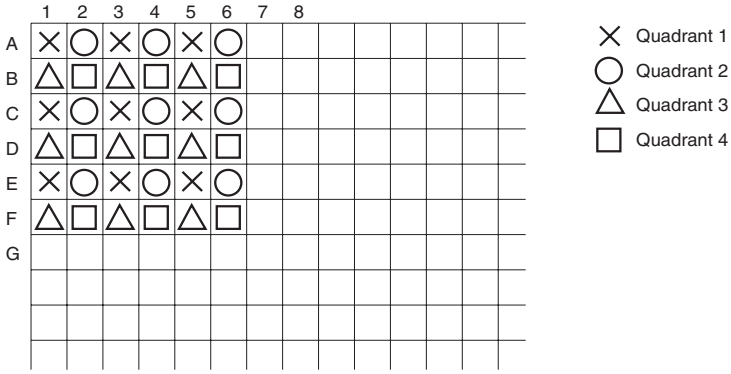
Item	Description
Diagnostic	Opens a menu that offers different diagnostic test dialog boxes. **Note** This option is only available to service personnel.
Remote Control	Opens the **Remote Control** dialog box that allows you to integrate a robotics system with the FLIPR system.
SMP Export	Opens an export dialog box that allows you to export a file in SOFTmax PRO format (*.smp).
Testers	Opens the **Task Tester** dialog box that allows testing of different system tasks. **Note** This option is only available to service personnel.

Graphs Menus

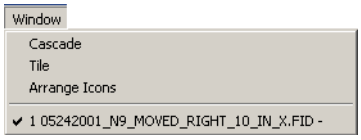


Item	Description
Single	Displays the data from a single well in the main graph. You can choose the well to be displayed by clicking the corresponding mini graph with the left mouse button.

Item	Description
Overlay	Displays all of the selected individual mini graphs in the main graph. You can select a series of mini graphs by dragging the cursor over the desired wells. Wells may also be added by selecting the row or column number. You can deselect the wells by right-clicking on the selected wells. To create a group, select the wells you want averaged. A group number is automatically assigned. Press the up-arrow key on the keyboard to move to the next group number and then select the desired wells for the next group. To go back to the previous group, press the down-arrow key
Average	Displays the average for a selected group of mini graphs in the main graph. You can create as many as 24 different groups for a 384-well plate and 12 groups for a 96-well plate. To create a group, select the wells you want averaged. A group number is automatically assigned. Press the up-arrow key on the keyboard to move to the next group number, and then select the desired wells for the next group. To go back to the previous group, press the down-arrow key.
Setup	Opens the **Graph Setup** dialog box which allows you to set up the graph display.
Auto Scale Main Graph	Automatically adjusts the Y-axis (fluorescence) scale to display all of the data taken in an experimental plate in the main graph. When enabled, it overrides any values specified in the **Maximum** and **Minimum** fields in the **Graph Setup** dialog box.
Auto Scale Mini Graph	Automatically adjusts the Y-axis (fluorescence) scale to display all of the data taken in an experimental plate in the mini graphs. When enabled, it overrides any values specified in the **Maximum** and **Minimum** fields in the **Graph Setup** dialog box.
Increment Group	When the **Display Selected** option has been chosen in the **Graph Setup** tab of the **Graph Setup** dialog box, this selects the next defined group of wells, displays it in the main graph and highlights it in the mini graphs. The current group's name is displayed in the **Status Bar**.
Decrement Group	When the **Display Selected** option has been chosen in the **Graph Setup** tab of the **Graph Setup** dialog box, this selects the previously defined group of wells, displays it in the main graph and highlights it in the mini graphs. The current group's name is displayed in the **Status Bar**.
Reset groups	This allows you to erase all of the previously defined groups and start over.
Group	This allows you to assign a specific group number to a selected group of wells and to choose which of the groups to highlight in the graphs.

Item	Description
Quadrant	The **Quadrant** sub-menu allows you to choose an individual quadrant and display its data in the mini graph window. To display all four quadrants, select **All**. **Note** This option is only available when using a 384-well cell plate. ♦ **Quadrant 1**—Starts with the lowest (first) odd-numbered column and row A, and then displays every other row and column. ♦ **Quadrant 2**—Starts with the lowest (first) even-numbered column and row A, and then displays every other row and column. ♦ **Quadrant 3**—Starts with the lowest (first) odd-numbered column and row B, and then displays every other row and column. ♦ **Quadrant 4**—Starts with the lowest (first) even-numbered column and row B, and then displays every other row and column. Shown below is a representation of quadrants in a 384-well plate. 

Window Menu



Item	Description
Cascade	Aligns all of the open windows so that they are overlapped and staggered with the title bars visible.
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.
Arrange Icons	Orders open data windows that have been minimized.
1–10 Data Files	Lists the open data files (up to10), along with the template file they were run with.

Help Menu

Item	Description
About FLIPR	Opens the **About FLIPR** dialog box that provides information regarding the version of FLIPR software that is currently installed on the system.
FLIPR User Manual	Opens an on-screen, searchable version of the *FLIPR² and FLIPR³ User Manual*.

FLIPR Software Toolbar

Toolbar Icons

Each button on the toolbar represents a menu command as a shortcut to activating that command.

Button	Name	Description
	Use default control file	Opens a new control file that has the default settings.
	Open control file	Opens the **Open Control File** dialog box that allows you to browse and open a FCF file type.
	Save control file	Opens the **Save Control File** dialog box that allows you to save the control file in the desired location.
	Load data file	Opens the **Load Data File** dialog box that allows you to browse and open a FID or FWD file type.
	Save data file	Saves the data file that is currently open and showing on-screen.
	Print	Opens the **Print** dialog box.
	Export Time Sequence	Opens an **Export** dialog box that allows you to export the time sequence data as a *.squ file type.
	Export Statistics	Opens an **Export** dialog box that allows you to export the statistics data as a *.stat file type.
	Setup Graph	Opens the **Setup Graph** dialog box that allows you to modify graph display settings.
	Single Well Graph	Displays data in the main graph that were selected from a single well in the mini graph.
	Overlay Graph	Overlays data in the main graph that were selected from multiple wells in the mini graph.
	Average Graph	Changes the main graph display so that it is showing averaged data from multiple wells that were selected in the mini graph.
	Scale Main Graph	Changes the maximum and minimum values of the X- and Y-axes in order to include all of the data points in the main graph display.
	Scale Mini Graph	Changes the maximum and minimum values of the X- and Y-axes in order to include all of the data points in the mini graph display.
	Zoom In Main Graph	Enlarges the area of data in the main graph, enabling you to view the data more closely.
	Zoom Out Main Graph	Diminishes the area of data in the main graph, enabling you to view more of the data at once.

Button	Name	Description
	Zoom In Mini Graph	Enlarges the area of data in the mini graph, enabling you to view the data more closely.
	Zoom Out Mini Graph	Diminishes the area of data in the mini graph, enabling you to view more of the data at once.
	Signal Test	Initiates a signal test of the instrument and then displays the results in the **Signal Test** dialog box. **Note** Available only when the instrument is connected and working.
	Define Experiment	Opens the **Experiment Setup** dialog box that allows you to set up an experiment and control file.
	Run Experiment	Starts the execution of the control file. **Note** Available only when the instrument is connected and working.
	Stop Experiment	Stops the execution of the control file. It is active only when an experiment is running.
	Open/Close Door	Acts like a toggle to open and close the main door of the instrument. **Note** Available only when the instrument is connected and working.

FLIPR Graphs

About the FLIPR Graph Displays

The FLIPR Graphs are used to display the real-time data from an experiment as it is carried out. The mini graphs display the data from all wells at once. The main graph is used to display the data from one or more wells that you have selected from the mini graph.

Note Selected mini graphs appear red when selected. When the pointer is over a selected mini graph, the trace will appear blue in the main graph.

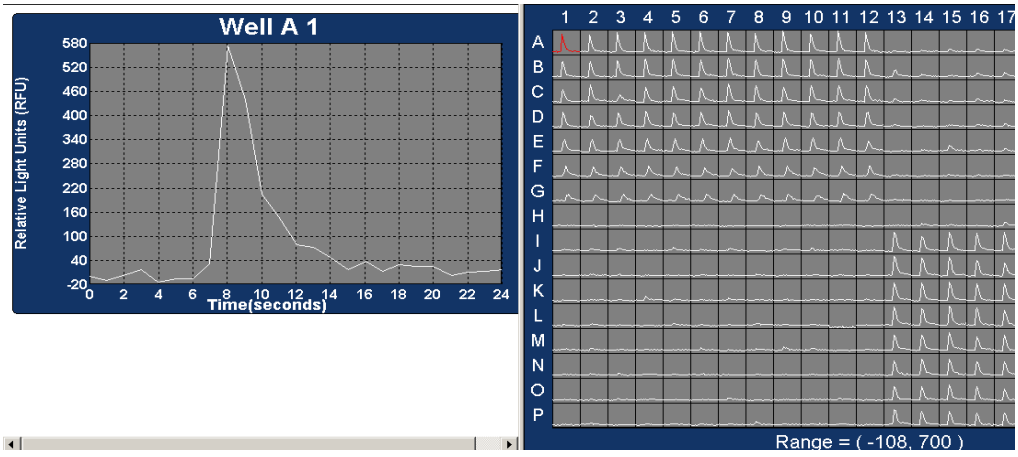
Main Graph Display

The data in the main graph can be modified to view:

- Data from a single well
- Data from selected multiple wells
- Average data of selected multiple wells

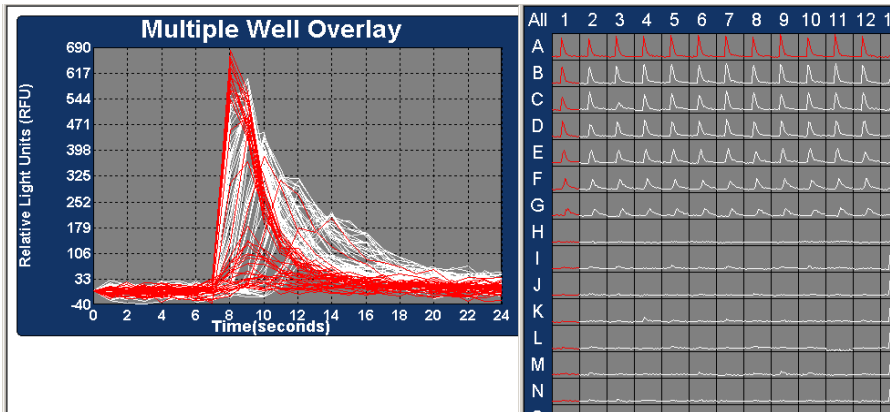
Single Well View

To view a single well, click on the well of interest in the mini graph. It will then be displayed in the main graph.



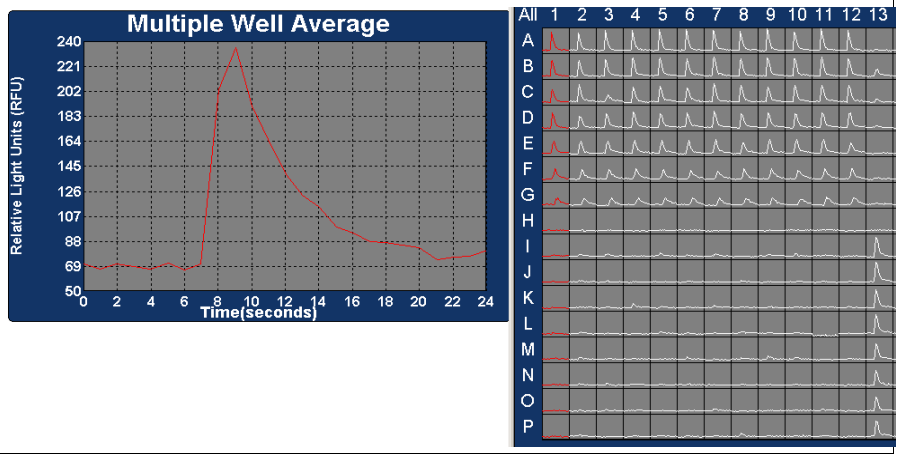
Overlay View

To view multiple wells in the main graph:

Step	Action
1	Select **Overlay** from the **Graphs** menu.
2	In the mini graph display, select multiple graphs by doing one of the following: ♦ Click and drag over the wells you want to view. ♦ Click a row or column head to view the entire row or column. Right-click the selected wells, rows, or columns to deselect them from view. 

Average View

To view the average data from selected multiple wells:

Step	Action
1	Select **Average** from the **Graphs** menu.
2	In the mini graph display, select the wells you want to average by doing one of the following: ♦ Click and drag on the wells you want to average. ♦ Click a row or column head to average the entire row or column. Right-click the wells, rows, or columns to deselect them from the average. 

Mini Graph Display

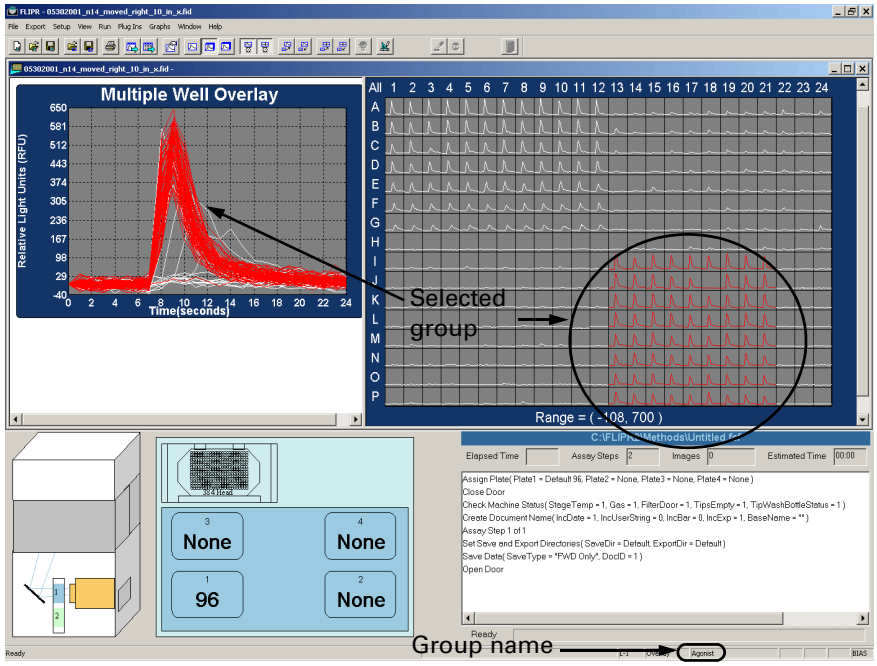
You can use the mini graph display to select and assign groups of wells that can then be displayed on the main graph using the overlay or average option.

Assigning Groups in the Mini Graphs

To assign groups:

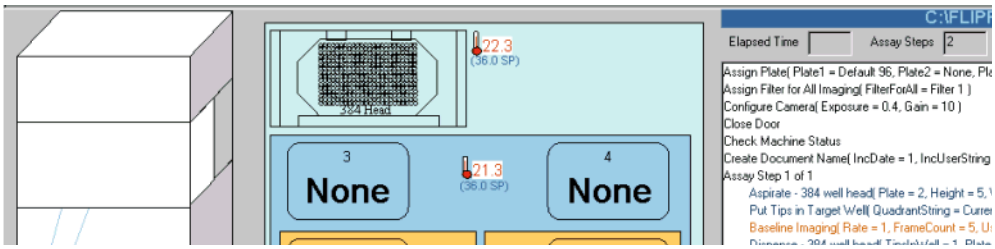
Step	Action
1	Select the desired graph view from the graph menu *i.e.*, **Average** or **Overlay**.
2	Select **Reset Groups** from the **Graphs** menu, if groups have already been chosen.
3	In the mini graph display, select the wells you want included in group 1 (as seen in **Overlay** or **Average** view). In the status bar on the bottom of the window, **Group 1** should be displayed. Alternatively, instead of pressing the up-arrow key, you may choose **Increment** or **Decrement** from the **Graphs** menu.
4	Press the **up-arrow** key on the keyboard. This assigns the selected wells to group 1 and increments to the next group, *i.e.,* **Group 2** (which will now be displayed in the status bar).
5	Select the wells you want to include in group 2 and press the **up-arrow** key to assign those wells to group 2 and move to **Group 3**.

To assign groups: *(continued)*

Step	Action
6	You may also assign names to the groups. a. Select **Setup** from the **Graphs** menu and click the **Group Names** tab. b. Enter the desired name in the field that corresponds to the group. c. Click **OK** to save the changes. Instead of the group number, the name will be displayed in the status bar. In the example below, the selected group has been named **Agonist**. 

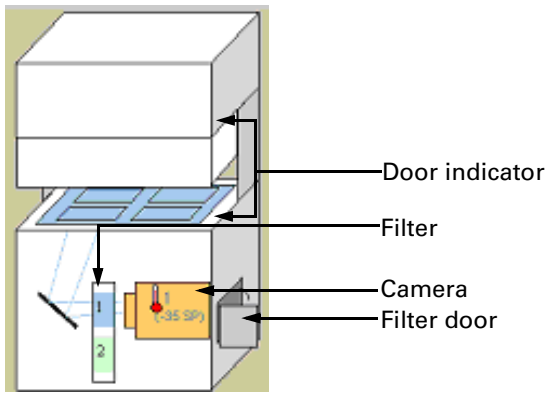
FLIPR Status Bar

FLIPR Status Bar Example



The **FLIPR Status Bar** is found near the bottom of the main window and displays the current status of the system's hardware components, stage, and experimental run.

Hardware Components



Above is a schematic of the instrument that indicates the present state of the following components:

Component	Description
Door	Displays the static and in motion state of the door —**open/closed/in motion/malfunction**
Filter	Displays which filter is in use, filter 1 or filter 2.
Camera	Displays the camera temperature
Filter door	Displays the state of the filter door–open/closed

Stage

The stage displayed in the **FLIPR Status Bar** indicates the current state (either static or in motion) of the pipettor and plates.

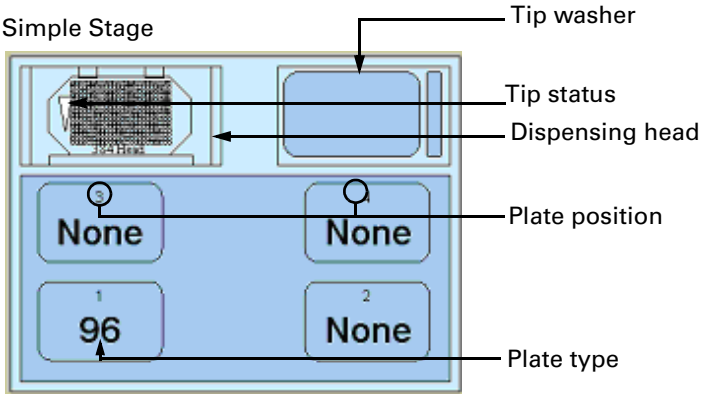
There are three types of stages:

- **Simple**—Used for running single plates at ambient temperature.
- **Heated**—Used to keep the plates at a constant temperature above ambient temperature.
- **Stacker**—Used to run multiple plates in one experiment.

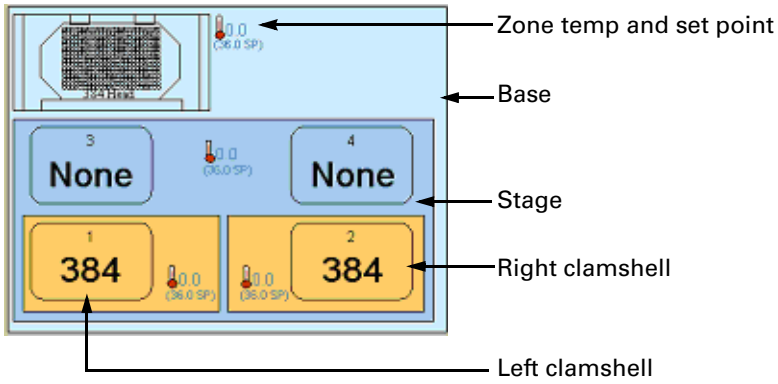
The stage on the **FLIPR Status Bar** indicates the following:

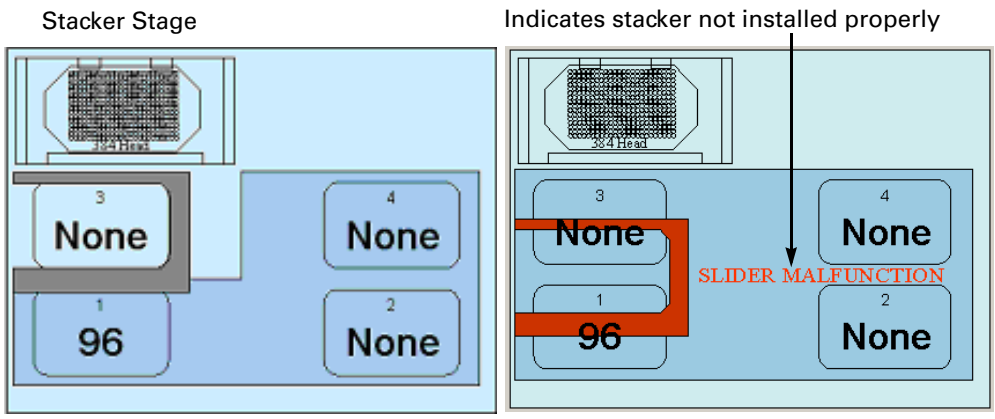
- Current dispensing head and its location
- Tip status (triangle present if tips present, blue triangle indicates tips have fluid)
- Tip washer status
- Plate position
- Plate type
- Stage temperatures (if using the heated stage indicators flash red when outside acceptable range)
- Stacker slider position (if using the stacker stage, see below)

Simple Stage

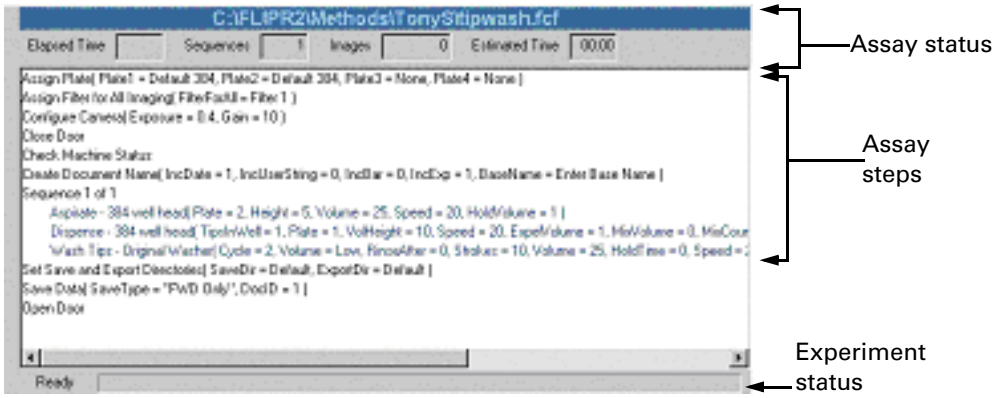


Heated Stage





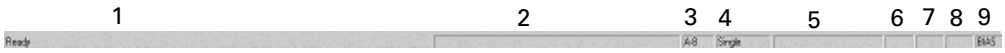
Experimental Run



This part of the **Status Bar** provides the following information:

Item	Description
Assay status	Indicates the progress of the assay by tracking the experimental parameters and displaying their values in real time. **Elapsed Time**—Amount of time the experiment has been running. **Assay Steps**—Assay step that is currently being executed. **Images**—Number of images that have been taken at the current elapsed time. **Estimated Time**—First time the experiment is run, this value remains zero. In subsequent runs, the estimated time will display the total elapsed time of the previous run.
Assay steps	A chronological list of all the assay steps in the control file.
Experiment status	Displays the state of the experiment *i.e.*, **Ready**, or **Running** and a bar that increments with the progress of the run.

Status Bar



The **Status Bar** is found at the bottom of the FLIPR software screen and displays information about whatever is currently taking place in the FLIPR system.

The **Status Bar** is divided into nine sections:

|Action Area (1) |Data Value (2) |Well Position (3) |Graph Type (4) |Selected Group (5) |Positive Control (6) |Negative Control (7) |Spatial Correction (8) |Bias (9)|

Item	Description
Action Area (1)	Displays the current state of the software or tip text.
Data Value (2)	Displays the value of the data point the cursor is directly over in the main graph, when the main graph is in **Single** mode.
Well Position (3)	Displays the well position of the selected mini graph.
Graph Type (4)	Displays the mode of the main graph *i.e.*,**Single**, **Overlay**, or **Average**.
Selected Group (5)	If groups have been defined, the name of the selected group (red) in the mini graph is displayed here.
Graph Settings (6—9)	Displays graph settings chosen in the experimental setup *i.e.*, positive control, negative control, spatial correction, bias.

Dialog Boxes and Windows

Becoming Familiar with Dialog Boxes and Windows

If you've worked with other applications that run using Windows, you'll probably be familiar with the conventions in this section. For a more detailed discussion of windows and dialog boxes, see the Microsoft Windows User's Guide.

Dialog boxes and overlay windows appear when you choose certain menu items or click some command buttons. You will know when a command will cause a dialog box to appear because the command will have an ellipsis (...) appended to it.

A dialog box presents a list of options available for the selected command and asks you to type in information that the program requires to proceed. It has buttons at the bottom of the screen (typically **OK** and **Cancel**, for example) that cause your selections/entries to be enacted. You cannot proceed to other actions until you close a dialog box.

A window overlay is much like a dialog box except that it does not have buttons and must be closed by clicking in the close box in the upper-right corner of the window. Windows do not need to be closed to take other actions in the main window.

Following are some of the typical items in dialog boxes and windows used in the FLIPR software.

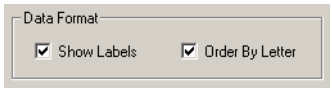
OK Button

The **OK** button carries through the choices you specify in the dialog box, closes the dialog box, and returns you to the software.

Cancel Button

The **Cancel** button causes the software to ignore any changes made in the dialog box at that point and returns you to the document window.

Check Boxes

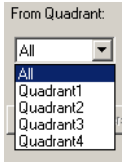


An enabled check box means that the option it corresponds to is turned on; a disabled check box means that it is turned off. A check box is enabled when an X or check mark appears in the check box and disabled when the check box is empty. If two or more check boxes appear together, you can enable one or all of the options contained within the grouping of the check boxes.

Option Buttons

An enabled option button means that the option it corresponds to is activated; a disabled option button means that it is inactivated. An option button is enabled when the circle next to the option is filled and disabled when the circle is empty. Option buttons are an either-or choice.

Drop-Down Menus

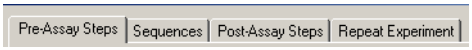


Drop-down menus contain multiple choices for a particular option and are activated by clicking on the arrow next to the option.

Text Field

Text fields require that you type in text.

Tabs



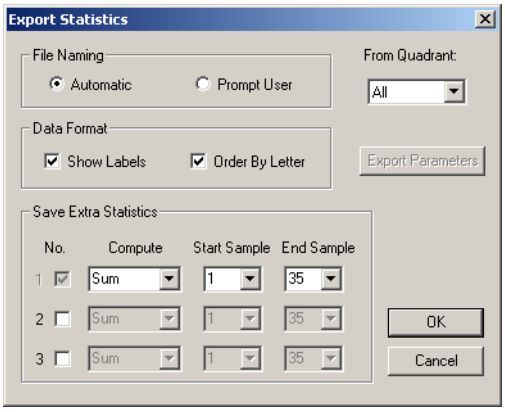
Tabs appear along the top of some dialog boxes. Tabs allow you to move between pages that group related commands together.

Export Menu Dialog boxes

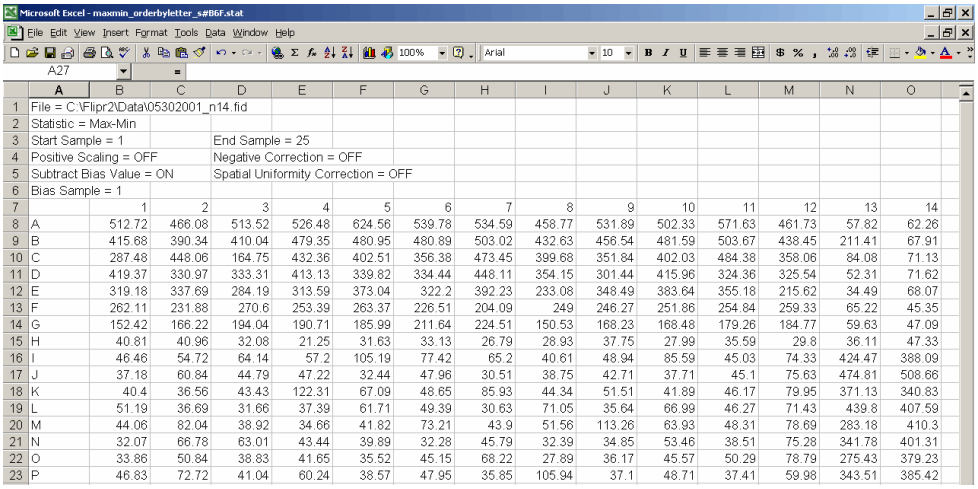
Statistics Dialog Box

Note FLIPR Operating Software version 2.X.X can only export information from data files created in version 2.0.14 or newer. Data created in FLIPR Operating Software version 1.26 or earlier can be viewed but not exported by the version 2.X.X software.

The **Export Statistics** dialog box, located under the **Export** menu, displays the settings that will be applied to a data file and will export the statistics data as an ASCII file. The statistics data file contains one data point per well.



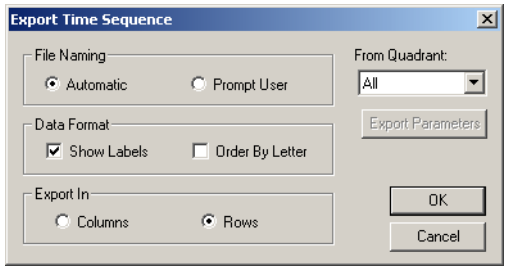
Item	Description
File Naming	When exporting a data file, this option allows you to choose automatic file naming or a prompt to enter a file name manually. When **Automatic** file naming is selected, the software names files using the following form: Filename_nX.stat, where X represents the number of the statistic (when greater than 1). When **Prompt User** is selected, you can name the exported file.
Data Format	The default data format is **Show Labels** and the resulting output file will contain information about the processing options selected (the name of the *.fid file and the well labels). Any data handling done prior to exporting the data (*e.g.*, negative control correction) is indicated in text format at the top of the exported ASCII file. If **Order by Letter** is checked, the columns in the exported file will be ordered A1, A2, A3; otherwise, the order will be A1, B1, C1. This option should be set according to the way the replicate wells are consecutive in the spreadsheet or database to ensure easy data handling. An example of an exported file ordered by letter is shown below.

Item	Description
Exported Statistic File 	
Save Extra Statistics	The **No.1, 2, 3** corresponds to saving three different statistics from the same data set. The file name of the second statistic saved will be Filename_nX.stat2, and the third will be stat3. The **Compute** field contains options that allow you to compute the data in five different ways: ♦ **Sum**—Numerical sum of the fluorescence counts of the selected images. Similar to area under the curve. ♦ **Average**—Numerical average of the fluorescence counts of the selected images. ♦ **Min**—Lowest fluorescence count (a single number) of all the images selected. ♦ **Max**—Highest fluorescence count (a single number) of all of the images selected. ♦ **Max-Min**—Result of subtracting the minimum fluorescence count (a single number) from the maximum fluorescence count (a single number).
From Quadrant	This panel allows you to choose a specific quadrant from which to export data, or you can choose to export data from all quadrants. It allows you to export data from one plate into four different spreadsheet files, if desired.
Export Parameters	This button opens the **Export Parameters** dialog box which enables you to select experimental parameters to export with the data.

Time Sequence Dialog Box

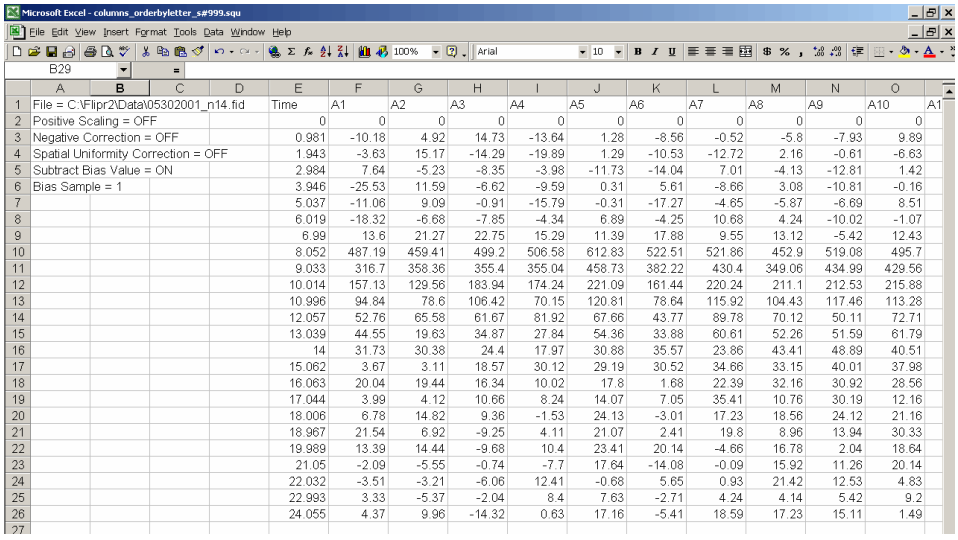
The **Export Time Sequence** dialog box, located under the **Export** menu, displays the settings used to determine file export parameters of a time sequence from a data file.

The main difference between **Time Sequence** and **Statistics** exports is that an exported time sequence file contains all of the data points for each well, whereas an exported statistics file contains one statistic per well.



Item	Description
File Naming	When exporting a data file, this option allows you to choose automatic file naming or a prompt to enter a file name manually. When automatic file naming is selected, the software names files using the following form: Filename_nX.squ, where X represents the number of the sequence file.
Data Format	The default data format is **Show Labels** and the resulting output file will contain information about the processing options selected. If **Order by Letter** is checked, the columns in the exported file will be ordered A1, A2, A3; otherwise, the order will be A1, B1, C1. This option should be set according to the way the replicate wells are consecutive in the spreadsheet to ensure easy data handling. An example of an exported file ordered by letter is shown below.

Exported Sequence File



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	A1
1	File = C:\Flipr2\Data\05302001_n14.fid				Time	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	
2	Positive Scaling = OFF				0	0	0	0	0	0	0	0	0	0	0	
3	Negative Correction = OFF				0.981	-10.18	4.92	14.73	-13.64	1.28	-8.56	-0.52	-5.8	-7.93	9.89	
4	Spatial Uniformity Correction = OFF				1.943	-3.63	15.17	-14.29	-19.89	1.29	-10.53	-12.72	2.16	-0.61	-6.63	
5	Subtract Bias Value = ON				2.984	7.64	-5.23	-8.35	-3.98	-11.73	-14.04	7.01	-4.13	-12.81	1.42	
6	Bias Sample = 1				3.946	-25.53	11.59	-6.62	-9.59	0.31	5.61	-8.66	3.08	-10.81	-0.16	
7					5.037	-11.06	9.09	-0.91	-15.79	-0.31	-17.27	-4.65	-5.87	-6.69	8.51	
8					6.019	-18.32	-6.68	-7.85	-4.34	6.89	-4.25	10.68	4.24	-10.02	-1.07	
9					6.99	13.6	21.27	22.75	15.29	11.39	17.88	9.55	13.12	-5.42	12.43	
10					8.052	487.19	459.41	499.2	506.58	612.83	522.51	521.86	452.9	519.08	495.7	
11					9.033	316.7	358.36	355.4	355.04	458.73	382.22	430.4	349.06	434.99	429.56	
12					10.014	157.13	129.56	183.94	174.24	221.09	161.44	220.24	211.1	212.53	215.88	
13					10.996	94.84	78.6	106.42	70.15	120.81	78.64	115.92	104.43	117.46	113.28	
14					12.057	52.76	65.58	61.67	81.92	67.66	43.77	89.78	70.12	50.11	72.71	
15					13.039	44.55	19.63	34.87	27.84	54.36	33.88	60.61	52.26	51.59	61.79	
16					14	31.73	30.38	24.4	17.97	30.88	35.57	23.86	43.41	48.89	40.51	
17					15.062	3.67	3.11	18.57	30.12	29.19	30.52	34.66	33.15	40.01	37.98	
18					16.063	20.04	19.44	16.34	10.02	17.8	1.68	22.39	32.16	30.92	28.56	
19					17.044	3.99	4.12	10.66	8.24	14.07	7.05	35.41	10.76	30.19	12.16	
20					18.006	6.78	14.82	9.36	-1.53	24.13	-3.01	17.23	18.56	24.12	21.16	
21					18.967	21.54	6.92	-9.25	4.11	21.07	2.41	19.8	8.96	13.94	30.33	
22					19.989	13.39	14.44	-9.68	10.4	23.41	20.14	-4.66	16.78	2.04	18.64	
23					21.05	-2.09	-5.55	-0.74	-7.7	17.64	-14.08	-0.09	15.92	11.26	20.14	
24					22.032	-3.51	-3.21	-6.06	12.41	-0.68	5.65	0.93	21.42	12.53	4.83	
25					22.993	3.33	-5.37	-2.04	8.4	7.63	-2.71	4.24	4.14	5.42	9.2	
26					24.055	4.37	9.96	-14.32	0.63	17.16	-5.41	18.59	17.23	15.11	1.49	
27																

Export In	This option allows you to choose whether to export the data in column or row format. Because the number of columns is limiting, if you are using a 384 format then you should export the data as rows.
From Quadrant	Allows you to specify from which quadrant to export the data, or you can choose to export data from all quadrants.

Batch Export Dialog Box

Batch export (statistics or time sequence) provides the same capabilities as the individual export functions in addition to exporting a number of data files at the same time.

Naming an export **Automatic** will export **X number** of export files for **X number** of data files.

Selecting **Prompt User** for file naming will export one export file that contains **X number** of data files. **Select Files** is then used to determine which files to export from a common directory.

To select multiple data files, use the **SHIFT** and **CTRL** keys to select the data files to be exported from the **Browse** dialog box. Selecting files from a common base name or type can be done using the wildcard key ***** to display only the desired files and then selecting from among them.

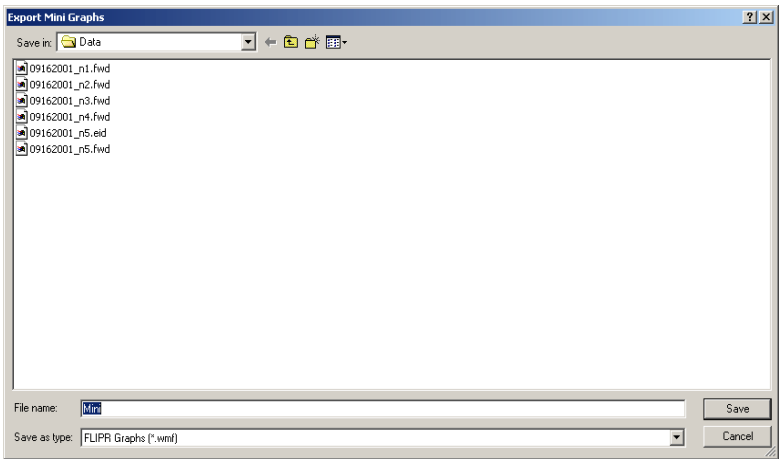
Note All data files must be closed prior to selecting the batch export function.

Main and Mini Graph and Control Export Dialog Boxes

The **Mini** and **Main Graph** export dialog boxes allow you to export these graphs as *.wmf files that can be imported into other software programs.

The **Export Control File** dialog box allows you to export a control file that can be opened in a spreadsheet program such as Microsoft Excel®.

Since these export dialog boxes are similar, only one is shown as an example.



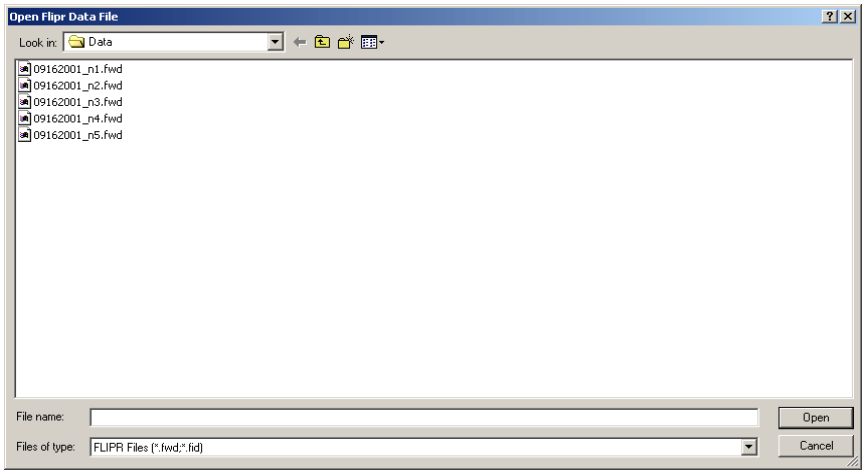
Item	Description
Save in	Designates the directory and file location for the exported graph.
File name	Text field for entering name of the exported file.
Save as type	Appends the file with a file type of *.wmf.

File Menu Dialog Boxes

Open Control or Data File Dialog Boxes

These dialog boxes appear when you choose **Open Control File** or **Open Data File** from the **File** menu. They allow you to open saved data or control files.

The dialog box for opening a data file is shown as an example

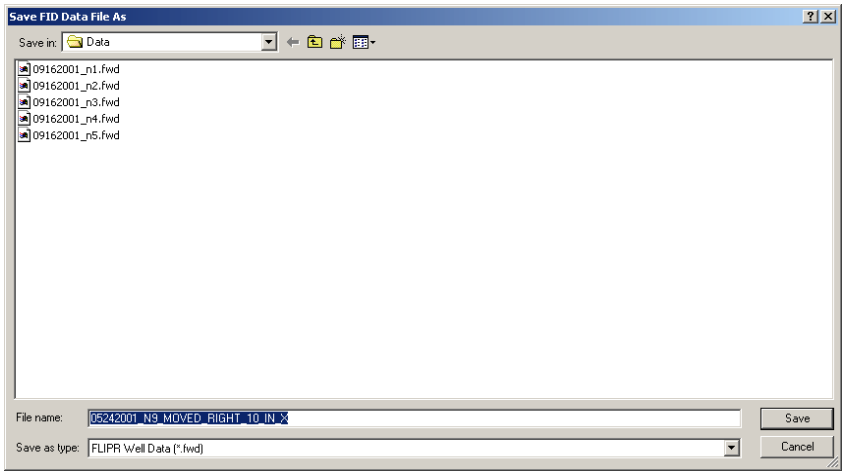


Item	Description
Look in	Allows you to browse the computer directories to locate the desired file.
File name	Displays the file name of the selected file.
File of type	Describes the type of files that will appear in the window. Control files have the *.fcf format and data files have a *.fid or *.fwd format.

Save Control or Data File As

These dialog boxes appear when you choose **Save Control File As...** or **Save Data File As...** from the **File** menu. They allow you to save a control or data file with a new name.

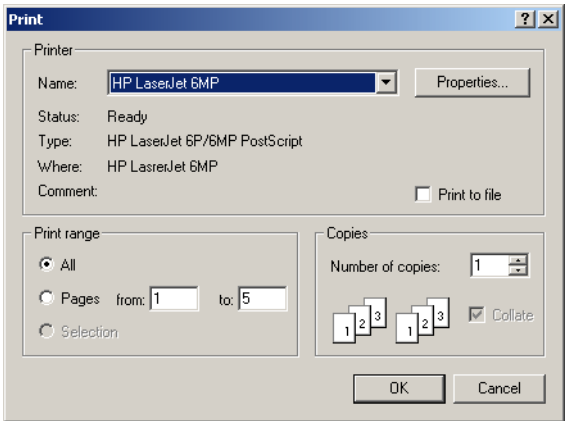
The dialog box for saving a data file is shown as an example.



Item	Description
Save in	Allows you to browse the computer directories and locate the desired folder for saving the file.
File name	Displays the file name of the selected file.
File of type	Describes the type of files that will appear in the window. Control files have the *.fcf format, and data files have a *.fid or *fwd format.

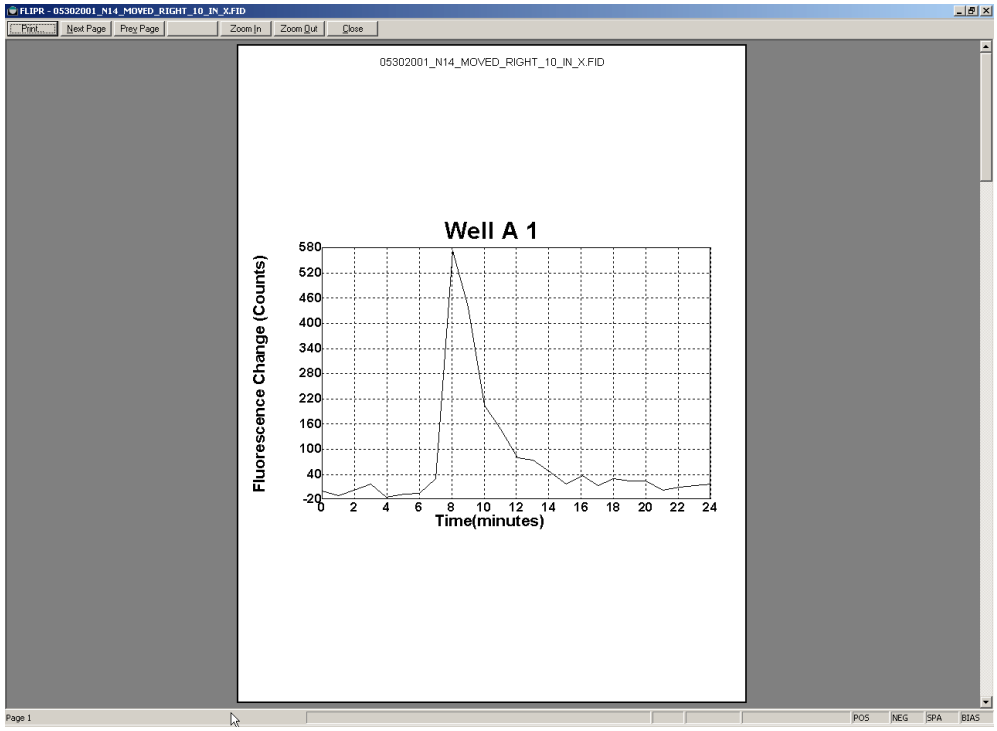
Print Dialog Box

This dialog box appears when you choose **Print** from the **File** menu. It allows you to select a printer and print part or all of the file that is currently open.



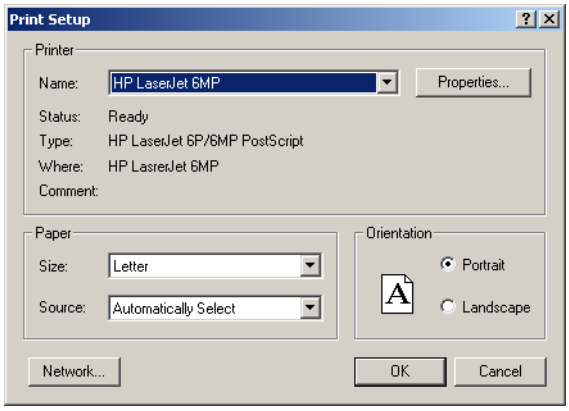
Print Preview Window

This window appears when you choose **Print Preview** from the **File** menu. It displays how your document will look when it is printed.



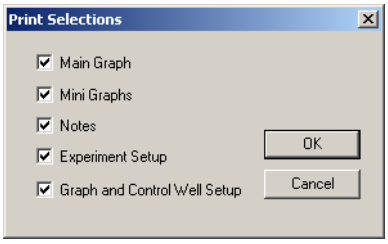
Print Setup Dialog Box

This dialog box appears when you choose **Print Setup** from the **File** menu. It allows you and designate the printer and the print parameters for your document.



Print Selections Dialog box

This dialog box appears when you choose **Print Selections** from the **File** menu. It allows you to choose what you want to print from the data or control file that you want to print.



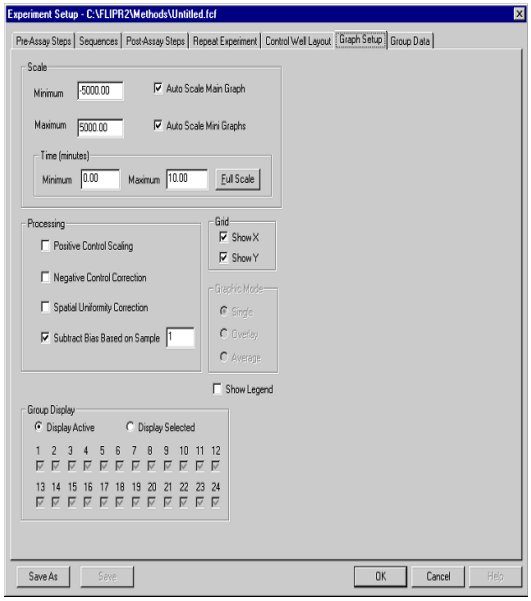
Graphs Menu Dialog Boxes

Setup Dialog Box

This dialog box appears when you choose **Setup** from the **Graphs** menu. It contains three tabs that allow you to modify settings and parameters for your data graph displays.

Graph Setup Tab

On this page you can set values and preferences for data graph display.



Item	Description
Scale	Allows you to choose how the Y-axis (relative luminescence units-RLU) and the X-axis (time) will be viewed. Most users choose to auto-scale the main graph (which displays only selected wells) and the mini graph (which displays all 384- or 96-wells at once) to start and set the **Time** panel to **Full Scale**. ♦ **Minimum**—Default value for this parameter is –5000, but it can be changed to any desired value. This parameter is applied if the **Auto Scale** box is not checked. ♦ **Maximum**—Default value for this parameter is 5000, but it can be changed to any desired value. This parameter is applied only if the **Auto Scale** box is not checked. ♦ **Auto Scale Main Graph**—Sets the fluorescence (Y-axis) scale to display all of the data taken in an experimental plate in the main graph. When enabled, it overrides any values specified in the **Maximum** and **Minimum** fields of the **Graph Setup** dialog box. ♦ **Auto Scale Mini Graph**—Sets the fluorescence (Y-axis) scale to display all of the data taken in an experimental plate in the mini graphs. When enabled, it overrides any values specified in the **Maximum** and **Minimum** fields of the **Graph Setup** dialog box.

Item	Description
Time	Allows you to set limits for the X-axis (time), if desired. The default is full scale. Clicking on **Full Scale**, after changing the X-axis, will reset the axis to the default.
Processing	The options in this panel allow you to manipulate the signal data in different ways. ♦ **Positive Control Scaling**—Averages the highest readings from the positive control wells and normalizes 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**—Averages each sample (reading) from the negative control wells and calculates the ratio of this value to the value of the first read. This ratio is calculated for each reading and applied to all the wells. This function provides a good correction for signal drift and artifacts. ♦ **Spatial Uniformity Correction**—Uses the initial signal to normalize the signal in each well, removing fluctuations due to well-to-well variation in cell density and dye loading. The processing algorithm takes the initial signal from all of the wells and averages them 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 the plate. **Note** Spatial uniformity correction is done in addition to calibration. ♦ **Subtract Bias Based on Sample**—Subtracts the fluorescence value measured at a selected sample (reading) from all the other time points in each well. It allows you to set the Y-axis scale so that at the specified time point, the Y-axis value for all of the data graphs is zero. **Note** The default is to select the first sample. To select another sample to subtract, check **Single** on the **Graphs** menu, then move the pointer to the graph and click the mouse. A cursor will appear in the graph. Use the left and right arrow keys to move the cursor to the desired sample. The **Status Bar** will display the position of the cursor *i.e.*, which sample it's in, the time, bias value, and the delta.The pointer will appear only when the **Graphs** menu is set to display a single graph. **IMPORTANT** The **Sample** field does not designate the time point for the desired subtraction, but the image number.
Grid	This panel controls the display of the horizontal and vertical grids in the main graph. ♦ **Show X**—Check this option to display the grid lines for the time (X) axis in the main graph. ♦ **Show Y**—Check this option to display grid lines for the fluorescence (Y) axis in the main graph.

Item	Description
Graphic Mode	This panel controls the display of graphic mode on the main graph. ♦ **Single**— Check this option to display the data from a single well. ♦ **Overlay**— Check this option to overlay data from multiple wells on the main graph. ♦ **Average**— Check this option to show the average graph for a selected group of wells.
Show Legend	When checked, this causes the main graph's legend to be displayed.
Group Display	This panel controls the display of groups you selected. The software allows data from individual wells to be grouped and plotted in the main graph. Up to 24 groups are allowed for 384-well plates and up to 12 groups for 96-well plates. Most users choose to display all of the groups by clicking on the **Display Selected** button. ♦ **Display Active**—When **Overlay** or **Average** is checked on the **Graphs** menu, only the active group (the current group selected in the mini graph) will be displayed on the main graph. ♦ **Display Selected**—When **Overlay** or **Average** is selected on the **Graphs** menu, all of the groups checked in the **Group Display** panel will be displayed concurrently. The active group (the group currently selected in the mini graph) will be displayed in red.

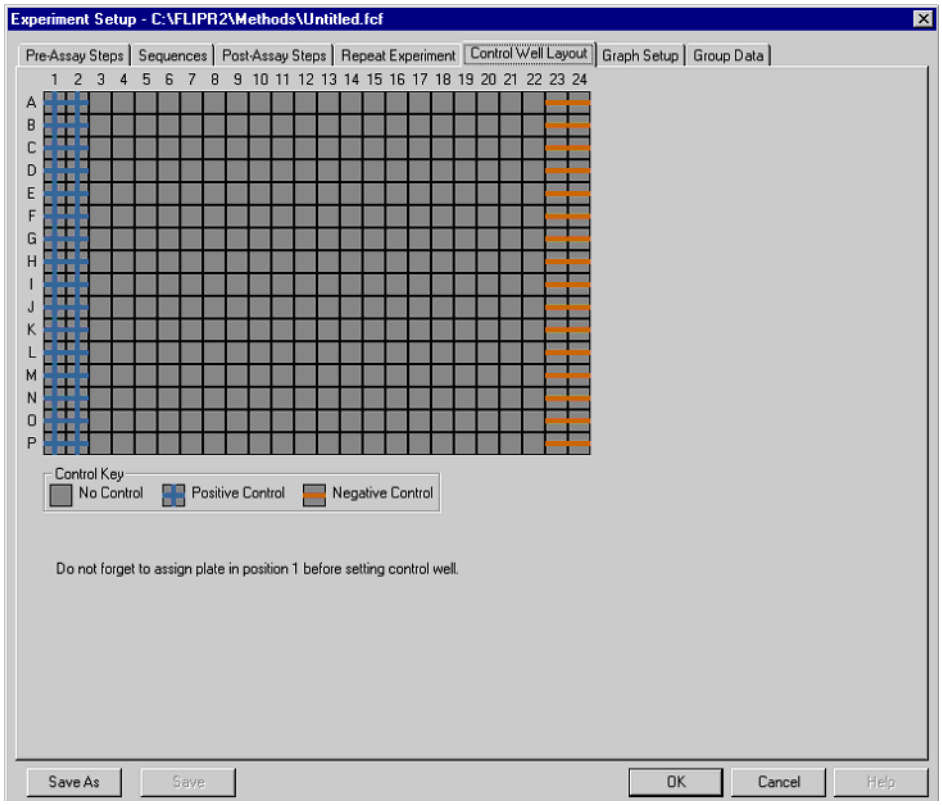
Control Well Layout Tab

In this dialog box, you can designate specific wells to be used as positive or negative controls.

Click on a well to designate it as a positive control well (typically the maximum response to a concentration of a given agonist),

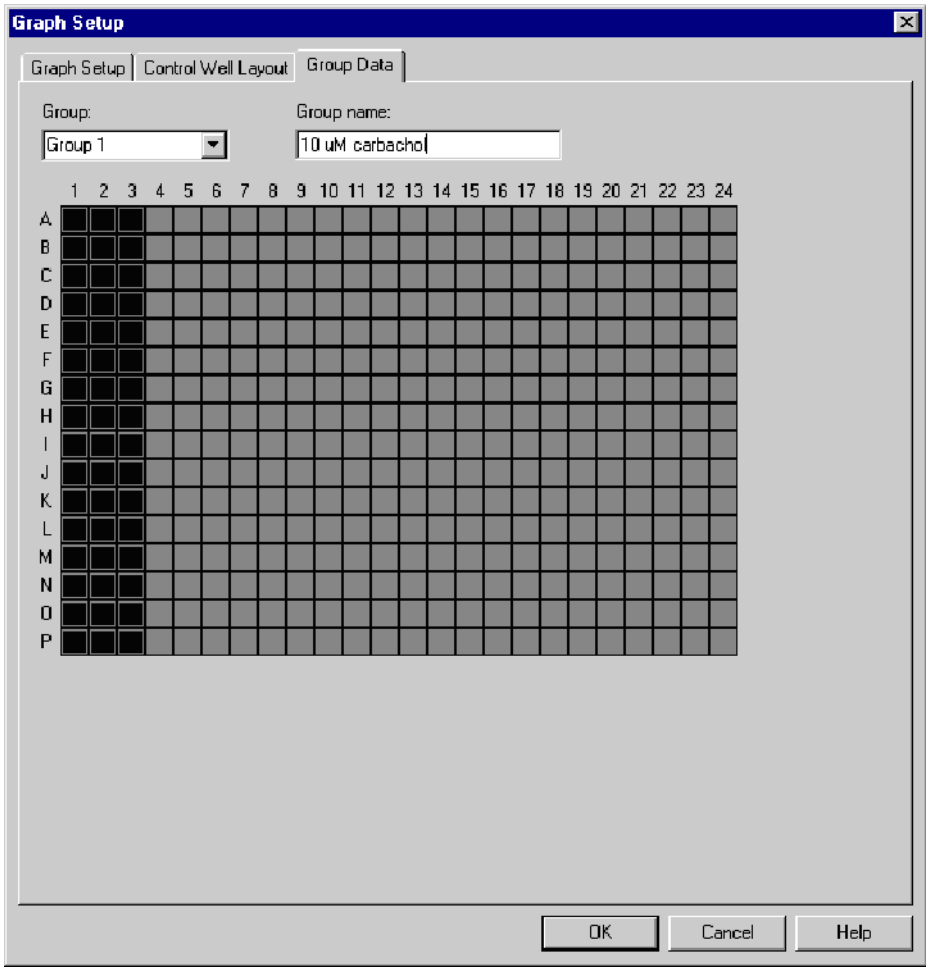
Double-click or right-click once on a well to designate it as a negative control (typically buffer addition controls).

You can deselect wells by double-clicking on the positive control wells and single-clicking on the negative control wells.



Group Data Tab

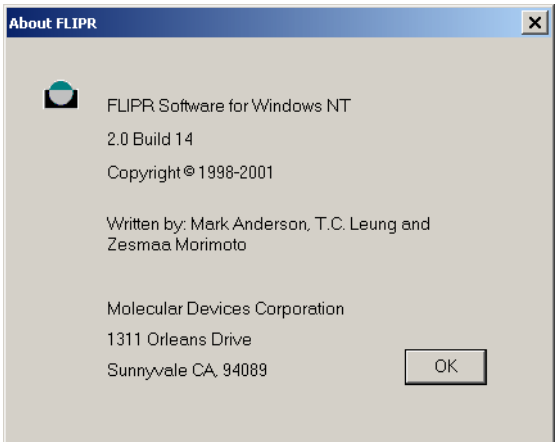
This dialog box is used to assign specific wells and a name to individual groups. When a group is displayed, the wells assigned to the group are highlighted in the mini graph and the name is displayed in the status bar.



Help Menu Dialog Boxes

About FLIPR Dialog box

This dialog box provides information about the current version of FLIPR software that is currently installed on your system.



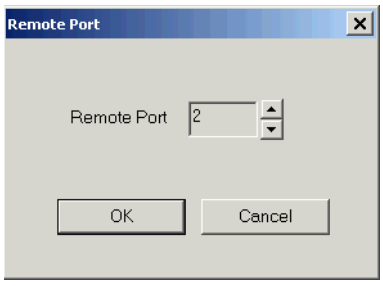
Plug Ins Menu Dialog Boxes (Optional)

About Plug Ins

These are additional features that are optional for purchase.

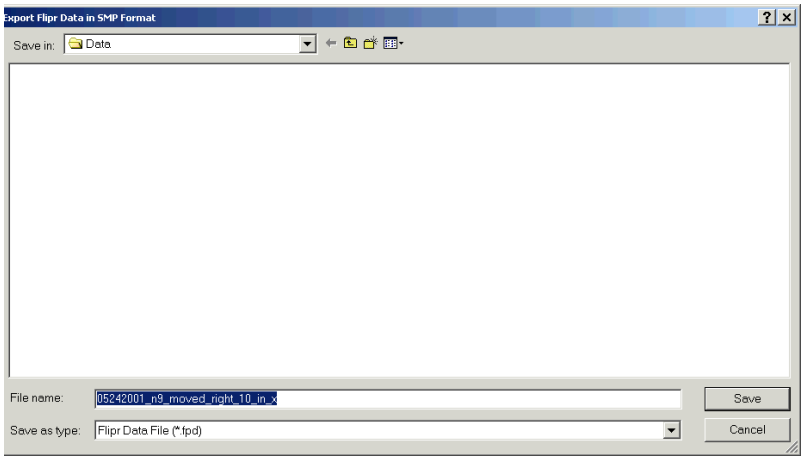
Remote Port Dialog Box

This dialog box appears when you point to **Remote Control** and select **On** from the **Plug Ins** menu. This dialog box allows you to integrate a robotics system with the FLIPR system.



SMP Export Dialog Box

This dialog box appears when you choose it from the **Plug Ins** menu. It allows you to export a file in the format (*.fpd) used in the SOFTmax PRO analysis software.



Run Menu Dialog Boxes and Windows

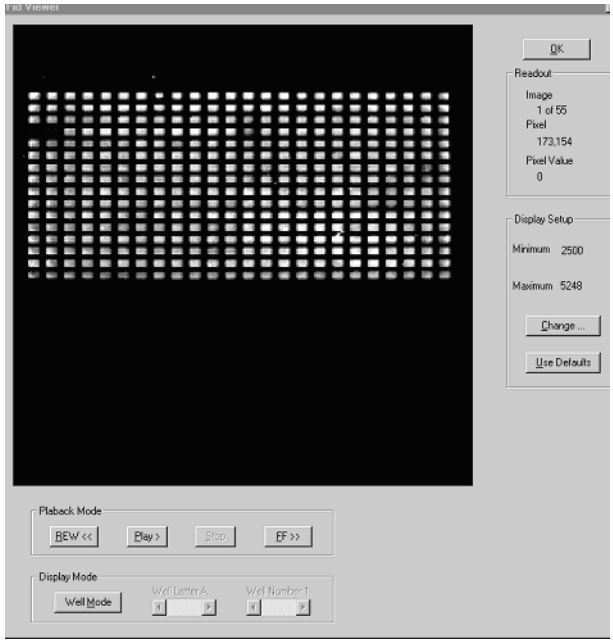
FID Viewer Window

This window appears when you select it from the **Run** menu. The **FID Viewer** allows you to review every image taken after an experiment, provided you have saved the data as a *.fid file, not as a *fwd file.

If you saved your experiment as a *.fid file, you may open the file with the **FID Viewer** and play back the sequential images taken during the experiment. You can view the sequence frame-by-frame or sequentially as a video.

The **FID Viewe**r can help you diagnose problems. For example, if the cells are blown off during fluid addition, the **FID Viewer** will display dark holes in the middle of the cell layer after addition of the compounds. If the entire well decreases in fluorescence, it is likely that extracellular dye has been diluted.

Note The **FID Viewer** image has been compressed from the initial picture intensity (which can be viewed using **Plate Viewer**) and therefore will have a lower resolution.

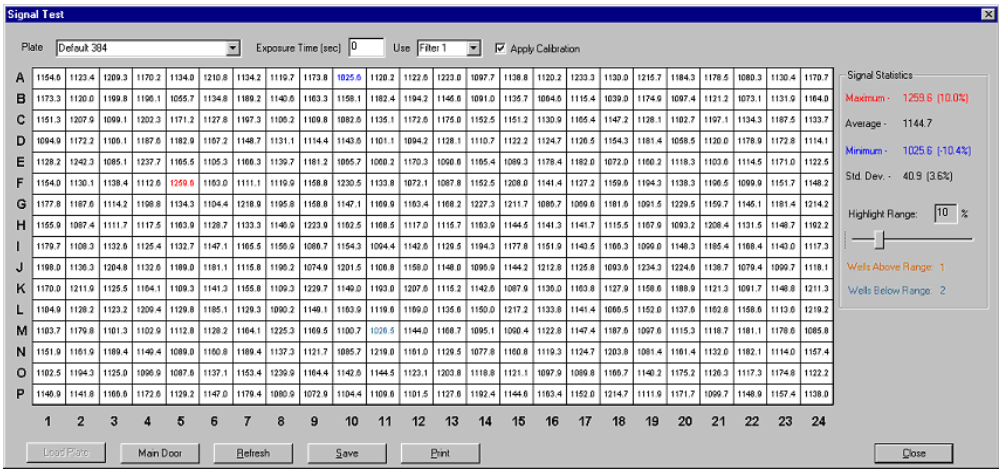


Item	Description
Readout	**Image**—The image number (out of the total number of images in the file) that is currently being viewed. **Pixel**—Out of the total number of pixels in the image, it displays the pixel number the arrow cursor is directly over. **Pixel Value**—Displays the value (fluorescence intensity) of the pixel the arrow cursor is directly over.

Item	Description
Display Setup	**Minimum**—Minimum range of the signal. **Maximum**—Maximum range of the signal. Clicking **Change** allows you to change the minimum and maximum range values. Anything below the minimum signal range will display as black, and anything above the maximum range will display as white. Everything within the signal's operating range will be displayed with a gray scale to indicate signal strength. Clicking **Use Defaults** resets the minimum and maximum range values to the defaults values.
Playback Mode	This panel allows you to control the playback of images. **REW**—Steps back one image at a time. **Play**—Scrolls through the images. **FF**—Steps forward one image at a time.
Display Mode	This panel allows you to move from **Full Mode** to **Well Mode**. In Full mode, the entire plate is displayed. In **Well Mode**, you may choose to zoom in on a particular well. Click the **Well Mode** button to activate the **Well LetterX** and **Well NumberX** options. Select the desired well using the left/right arrow keys next to the **Well LetterX** and **Well NumberX** fields. Click **Full Mode** to exit **Well Mode** and view the whole plate.

Signal Test Dialog Box

This dialog box appears when you select it from the **Run** menu. Selecting **Signal Test** causes the instrument to take a picture of the plate in the read position. The numerical results are then displayed in the **Signal Test** dialog box.



The signal test has two functions:

- Checking the state of the overall system
- Checking the initial fluorescence of a plate prior to running an assay

Item	Description
Plate	From the drop-down menu, choose the plate type you are using.
Exposure Time (sec)	Enter the amount of time you want the camera shutter to be open during the signal test.
Use	Select the appropriate filter to be used for the signal test.
Apply Calibration	Check this option if you want the calibration file to be applied to the results of the signal test.

Running a System Check

An overall system check is typically run once a day using a calibration yellow plate. When performing this test, make sure that the system is turned on and warmed up to ensure that the system is functioning properly.

For FLIPR^2 systems: using a 96- or 384-well yellow plate, set the exposure time to 0.05 sec, the laser power to 0.6 W, the camera f/stop to 2 and camera gain to 10. The desirable fluorescence counts for the signal test is 8,000–20,000 counts. For FLIPR^3 systems: use the same settings and set the gain to 10.

Running a Plate Prior to an Experiment

Run the signal test using the camera parameters as defined in the **Experiment Setup** dialog box. Depending on the range of values obtained, you can choose either to run the experiment or to alter the laser, exposure time, gain or camera f/stop settings.

A suggested starting point for a Ca^{2+} assay is 8,000–20,000 counts.

To change the settings, exit the signal test. The parameters that can be changed to optimize fluorescence are as follows:

- Laser power—Doubling the laser power doubles the counts.
- Exposure length—Doubling the exposure time doubles the counts.

Note With longer exposure times, you may not be able to take one sec updates.

- Gain–Increasing the gain will exponentially increase the counts.
- F-stop—Every increase in f-stop cuts the counts by one-half.

Change the settings as desired, return to the signal test and recheck the ranges.

Note Although the signals displayed may not reach the saturation value of 65,000 counts, the **Camera Saturation** error message will be displayed if there is potential saturation of the signal (signal saturation defined by pixels > 64,000 counts).

IMPORTANT The fluorescence signals displayed in the **Signal Test** dialog box are not saved as part of the data file.

Plate Viewer Dialog Box

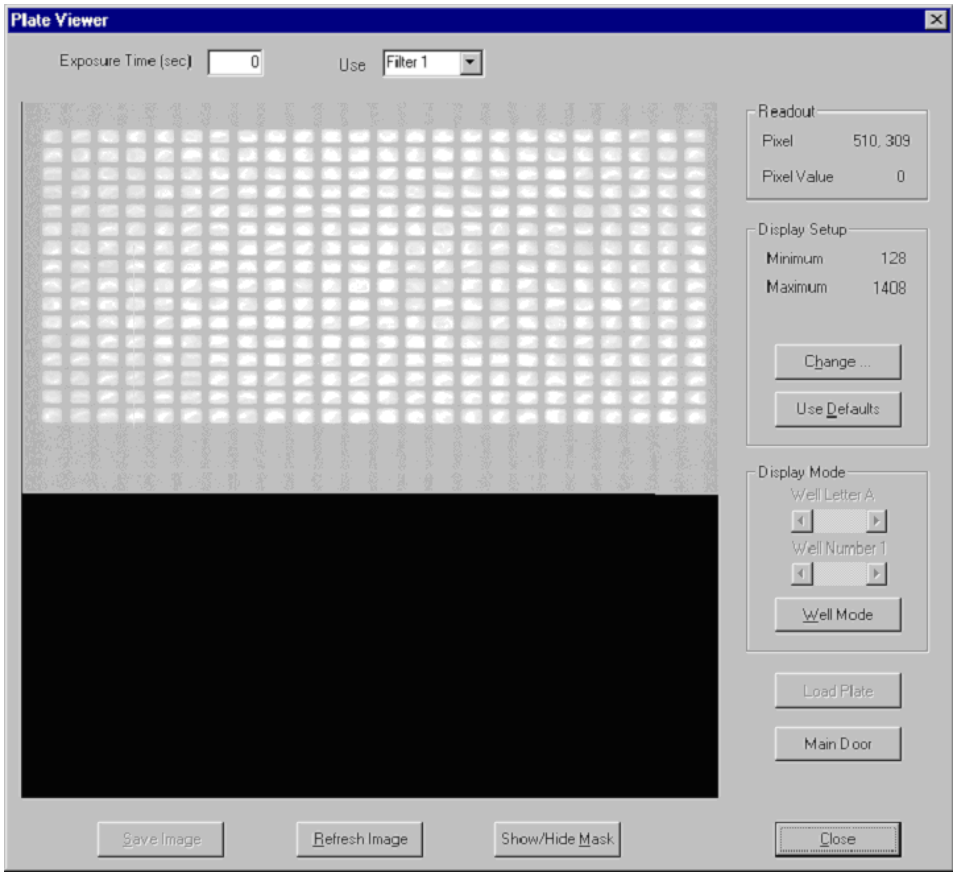
This dialog box appears when you choose **Plate Viewer** from the **Run** menu.

This option enables the FLIPR software to acquire an image of the current plate using the current exposure length and camera settings. This image is then displayed along with the current fluorescence signal.

The **Plate Viewer** is the picture equivalent to the fluorescent intensities obtained when running a signal test.

The **Plate Viewer** can help you diagnose problems with cells. If the cells are weakly adherent and removed from the read area of the well after the wash step, the **Plate Viewer** displays dark spots. This is an indication of low or no fluorescence.

IMPORTANT Although the image obtained using the **Plate Viewer** is not saved as part of the data file, it can be saved by clicking the **Save** button.

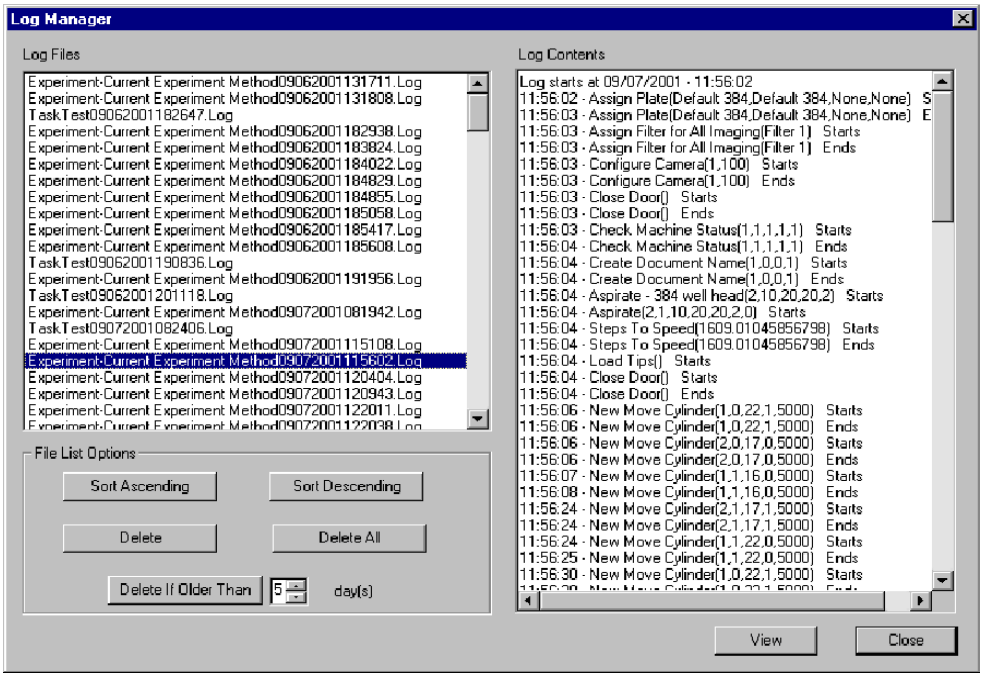


Item	Description
Readout	This panel displays the total number of pixels in the image and the pixel value of the arrow cursor's current position.
Display Setup	**Minimum**—Minimum range of the signal. **Maximum**—Maximum range of the signal. Clicking **Change** allows you to change the minimum and maximum range values. Anything below the minimum signal range will display as black, and anything above the maximum range will display as white. Everything within the signal's operating range will be displayed using a gray scale to indicate signal strength. Clicking **Use Defaults** resets the minimum and maximum range values to the defaults values.

Item	Description
Display Mode	This panel allows you to move from **Full Mode** to **Well Mode**. In **Full Mode**, the entire plate is displayed. In **Well Mode**, you may choose to zoom in on a particular well. Click the **Well Mode** button to activate the **Well LetterX** and **Well NumberX** options. Select the desired well using the left/right arrow keys next to the **Well LetterX** and **Well NumberX** fields. Click **Full Mode** to exit **Well Mode** and view the whole plate.
Load Plate	Clicking this button allows the user to load a plate into the read position using the stacker. This option is available only when the stacker is configured.
Main Door	A toggle that opens and closes the door to the instrument to allow you to remove or insert a plate.
Show/Hide Mask	Clicking the **Show/Hide Mask** button toggles the display between an image with and without the mask, respectively
Refresh Image	Retakes and displays another image using the current settings.
Save Image	Clicking this button opens the **Save as** dialog box which allows you to save the plate image. **Note** This option is not currently available.
Close	Clicking the **Close** button closes the **Plate Viewer** window.

Log Manager Window

This window appears when you choose **Log Manager** from the **Run** menu. The **Log Manager** is used to manage the log files. Log files contain a complete record of the executed experiment and are useful for troubleshooting. They have a file extension of *.log.



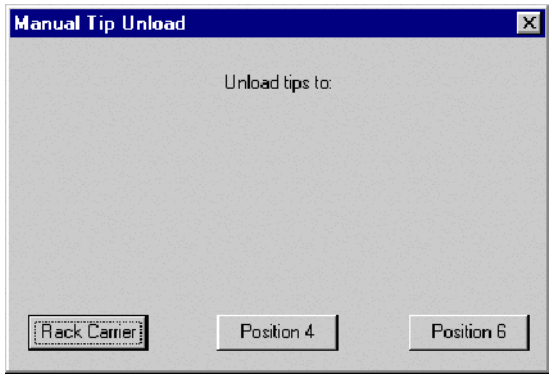
Item	Description
Log Files	Lists all of the stored log files
Log Contents	When a log file is selected, click the **View** button to view the contents of the log file in the **Log Contents** area. Double-clicking on the file will also display the log file contents.
File List Options	**Sort Ascending**—Alphabetically orders the log file list from top to bottom. **Sort Descending**—Alphabetically orders the log file list from bottom to top. **Delete**—Removes the selected log file from the Logs folder. **Delete All**—Removes all of the log files from the Logs folder. **Delete Files Older Than**—Deletes files older than X (1–9) number of days
View	When a log file is selected, click on **View** to display the contents of the file in the **Log Contents** area.

Manual Unload Tips

This dialog box appears when you choose **Manual Unload Tips** under **Special Operations** in the **Run** menu. This option allows you to unload tips to a location other than the load position, if desired.

Click on the position to which you want to unload.

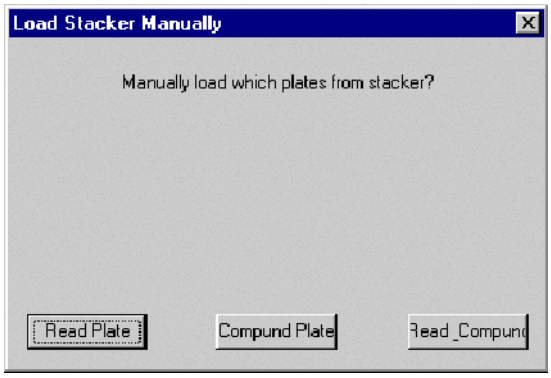
IMPORTANT If you do choose another location, make sure you have placed a container in the appropriate position to receive the tips.



Load Stacker Manually

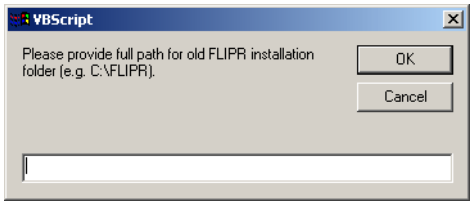
This series of dialog boxes appears when you choose **Load Stacker Manually** under **Special Operations** in the **Run** menu. Use this option if you want to run a plate manually from the stacker *e.g.*, to run a yellow plate test before a multiple plate run.

You will be prompted to choose which type of plate you want to read and from which location. For more information on using this feature to read a plate, see Chapter 6, *"Using The Plate Stacker."*



Convert Setup Files Dialog Box

This series of dialog boxes appears when you choose **Convert Setup Files** under **Special Operations** in the **Run** menu. It is used only during the installation of system software. See *"Creating a New Setup File"* on page 4-16 for more information.



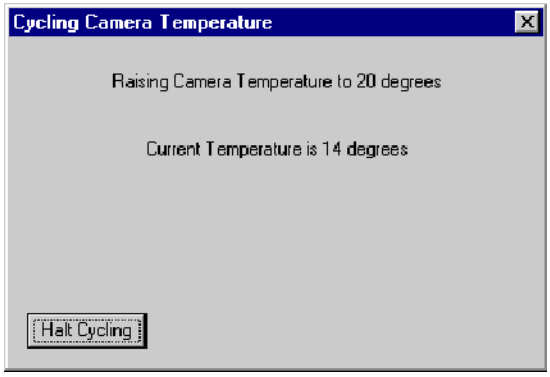
Initialize Tip Washer

This series of dialog boxes appears when you choose **Initialize Tip Washer** under **Special Operations** in the **Run** menu. This operation prepares the tip washer for use, and if you are using the tip washer, the **Initialize Tip Washer operation** should be executed prior to starting the experiment.

Cycle Camera Temperature

This dialog box appears when you choose **Cycle Camera Temperature** under **Special Operations** in the **Run** menu. It is used to reset the camera when you want to run low-fluorescence (*e.g.*, luminescence) experiments immediately after running high-fluorescence experiments.

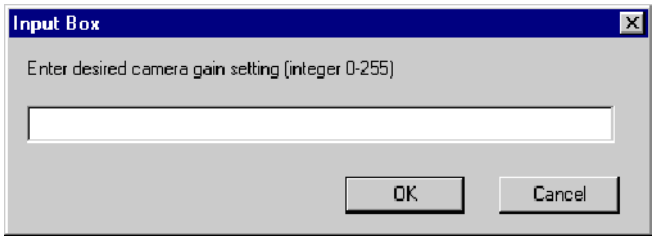
During the cycle period, the camera temperature will be raised to room temperature (20–25° C) and then cooled to –35° C in a time span of approximately 15 min.



Set Camera Gain

This dialog box appears when you choose **Set Camera Gain** under **Special Operations** in the **Run** menu. Use this feature when you want to set the camera gain manually.

To set a camera gain, enter an integer value between 0 and 255 in the text field and click **OK**.



Experiment Setup Dialog Box

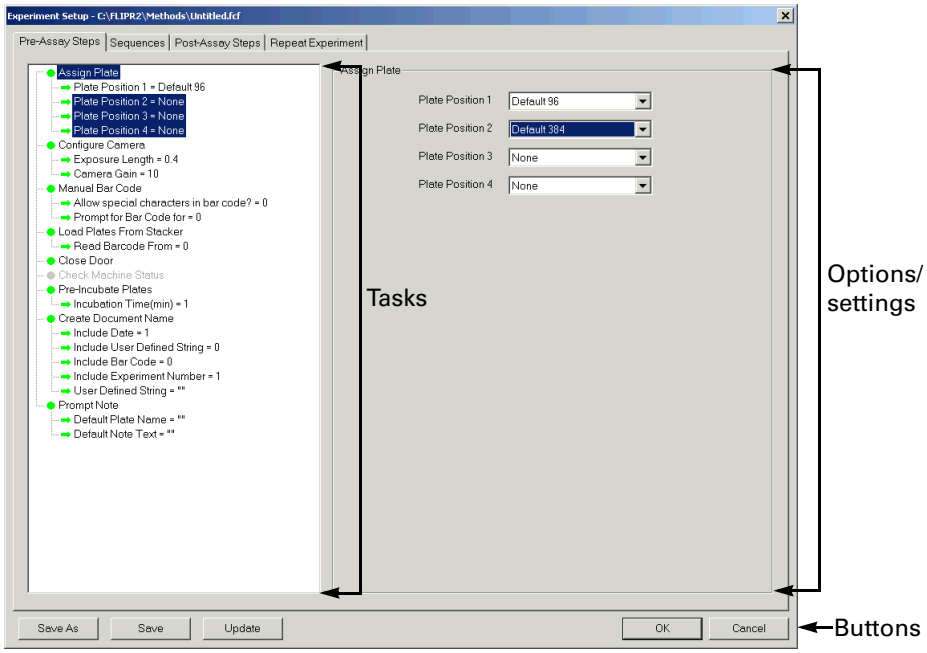
About the Experiment Setup Dialog Box

This dialog box appears when you choose **Experiment** from the **Setup** menu. It has four tabs of settings that enable you to control how the experiment is run and how the data is stored.

On the left side of each tab there is a list of tasks associated with that particular tab. The conventions used with these tasks are as follows:

- Enabled tasks have a green circle next to the them.
- Disabled tasks have a gray circle and the task is dimmed.
- Some tasks are optional. Double-clicking on an optional task that has a gray circle will activate it. Double-clicking on a green circle will deactivate it.
- Some tasks are required and thus always have a green circle next to them.
- Non-selectable tasks in the current mode of system operation will not appear on the task list.

To choose an option for a task, click on the task in the left area of the dialog box. This will display the options for that task on the right-side of the dialog box.



Buttons	Description
OK	Closes the dialog box with the current values. If there is an error in a selection, a warning dialog box will appear indicating where the error has occurred. You will not be able to close the dialog box until the error is corrected. However, you will be able to save the experiment setup, even with an error.
Cancel	Closes the dialog box without saving any new changes.

Buttons	Description
Save As	Opens the **Save Protocol** dialog box and allows you to change the name and save the experiment setup as a *.fcf control file.
Save	Saves the experiment setup as a *.fcf control file.
Update	Values entered in the right-side of the box are updated after you click **OK** or when you click **Update**. Clicking **Update** will also update the **FLIPR Status Bar**.

Minimum and Maximum Values for the Experimental Setup

The table below provides the minimum and maximum range that can be used in the experiment setup dialog box. Use this table as reference when you are creating a control file.

PreAssay Parameter	Range	Notes
Exposure length	0.05–60 sec	—
Camera gain	0–255	For FLIPR^3 systems, value must be an integer.
Pre-Soak time	0.1–60 min	—
Height (in plate for aspirate or dispense)	From 1/40 of full-well capacity to full-well capacity for a single well	Value must be an integer. Full-well capacity is defined in the Plate Library.
Aspirate volume	1–25 µL (for a 384-well head) 5–250 µL (for a 96-well head)	Value must be an integer.
Speed (for aspirate or dispense)	1 µL minimum. Maximum is determined by system setup (typically ~950 for a 96-well head, ~250 for a 384-well head).	Value must be an integer. Maximum varies slightly on different systems.
Expel Volume	0 µL minimum. Maximum determined by system setup (typically ~8 µL for a 96-well head, ~2 µL for a 384-well head).	Maximum varies slightly on different systems
Hold Volume	0–5 µL	Maximum tip volume cannot be exceeded.
Number of Images	1–100	Value must be an integer. Maximum number/experiment varies based on available memory in the system. Warning is given if experiment requested.

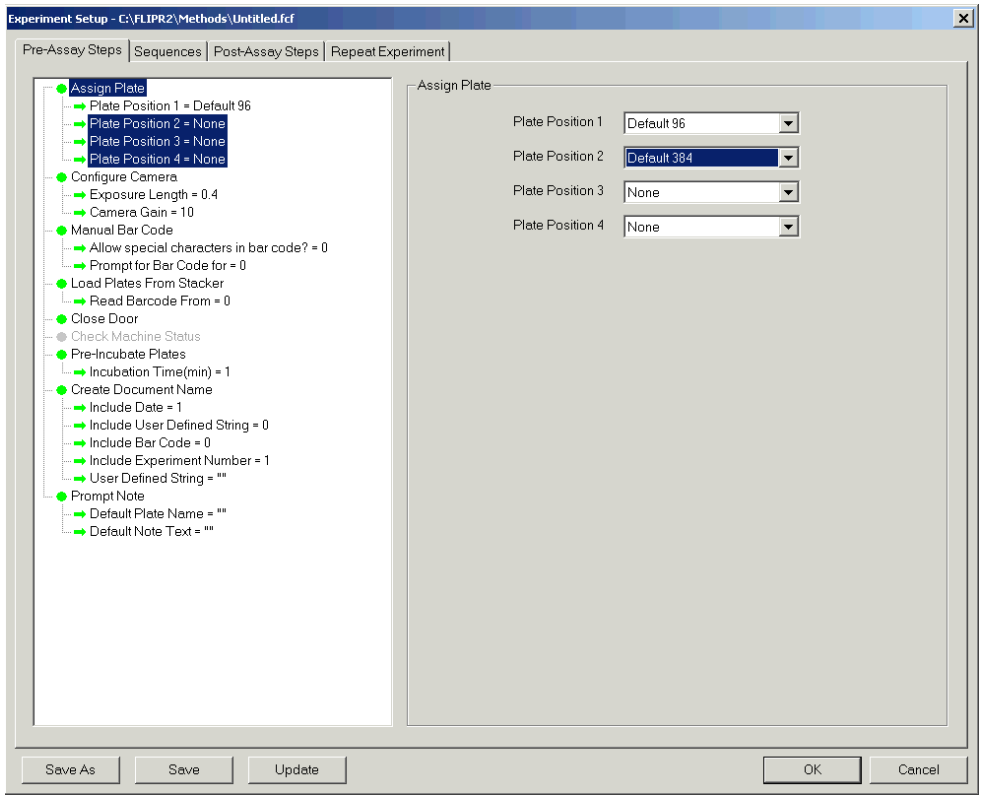
Pre-Assay Steps Tab

On this page of the dialog box, you may choose options associated with tasks that will be performed before the experiment starts *i.e.*, before compounds are added to the cells and images are taken.

Assign Plate

The **Assign Plate** task is selected in the figure below. The settings associated with this task are displayed in the **Assign Plate** panel.

The **Plate Positions (1–4)** correspond to the stage position (see *"FLIPR Status Bar Example"* on page 4-44). The choices for the plate positions are displayed in a drop-down menu next to each plate position and are derived from a list of default plates and additional plates that have been added to the software using the **Plate Library** dialog box.



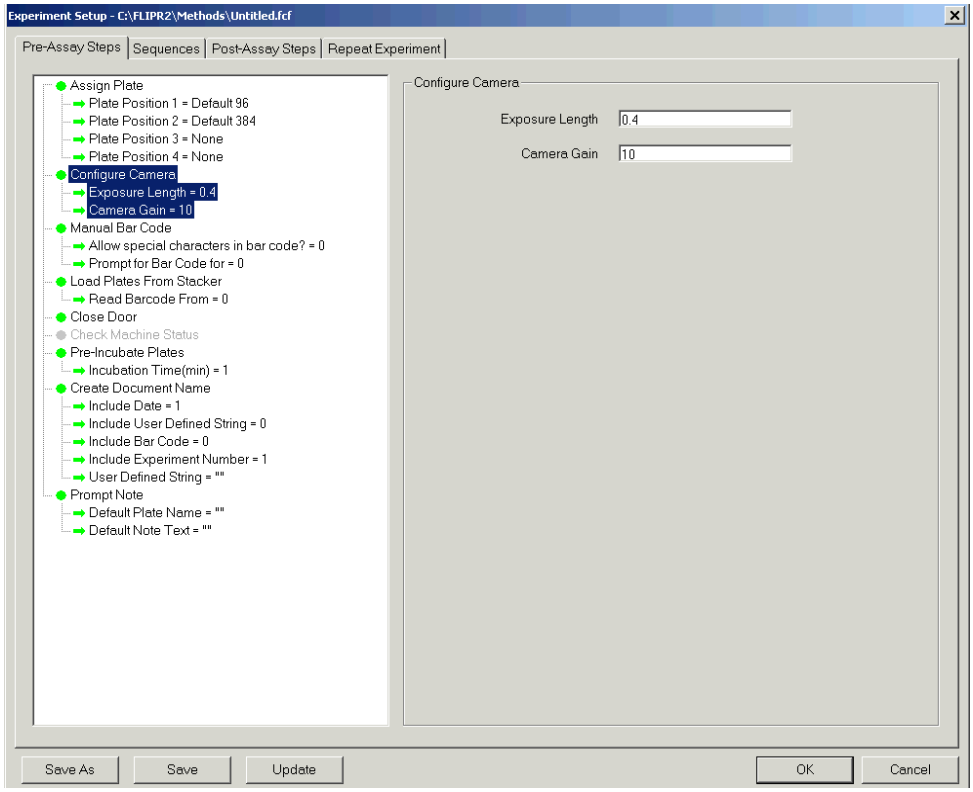
Configure Camera

The **Configure Camera** task is selected in the figure below. The settings allow you to enter an exposure time for each image and the camera gain.

The exposure time is the time (in seconds) that light will be collected and measured by the camera. The exposure time will influence the signal recorded. The minimum exposure time that can be used is 0.05 seconds. If you want to collect more light, increase the exposure time. The maximum exposure time is 60 seconds. The exposure length also determines the update time for the data collection. For FLIPR^2 systems, the camera requires 0.6 seconds to integrate the data from each exposure, and the highest update frequency for an exposure length of 0.4 seconds is once per second. For FLIPR^3 systems, the camera requires 0.3 seconds to integrate the data from each exposure, and the highest update frequency for an exposure length of 0.7 seconds is once per second.

The camera gain indicates the amplification of the camera power. Increasing the camera gain will increase the signal. **Camera Gain** has a range of 0–255.

Note The **Camera Gain** function is operational only on FLIPR^3 systems.



Assign Filter for All Imaging

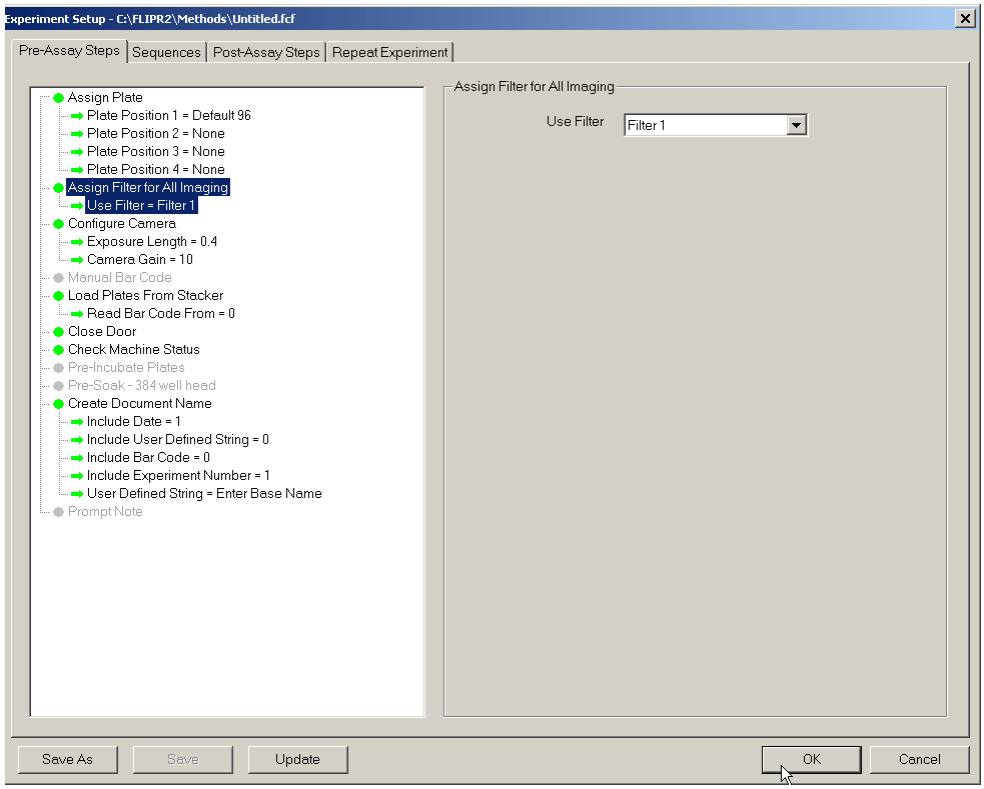
The **Assign Filter to All Imaging** task is selected in the figure below. The settings associated with this task can be modified in the **Use Filter** option of the **Assign Filter for All Imaging** panel.

Click on the drop-down menu arrow of **Use Filter** and select a filter.

Note This setting changes the filter location in the instrument and on the FLIPR status bar when you click **OK**.

Most of the standard fluorescence dyes used with the FLIPR system will work with **Filter 1**. This filter has a bandpass of 510–570 nm. For dyes that emit at other wavelengths, an additional filter can be placed in the **Filter 2** slot. Please call Molecular Devices for specific information on other filters.

Note If **Both** is selected, the experiment will alternate filters and create two separate data files, one for each filter.

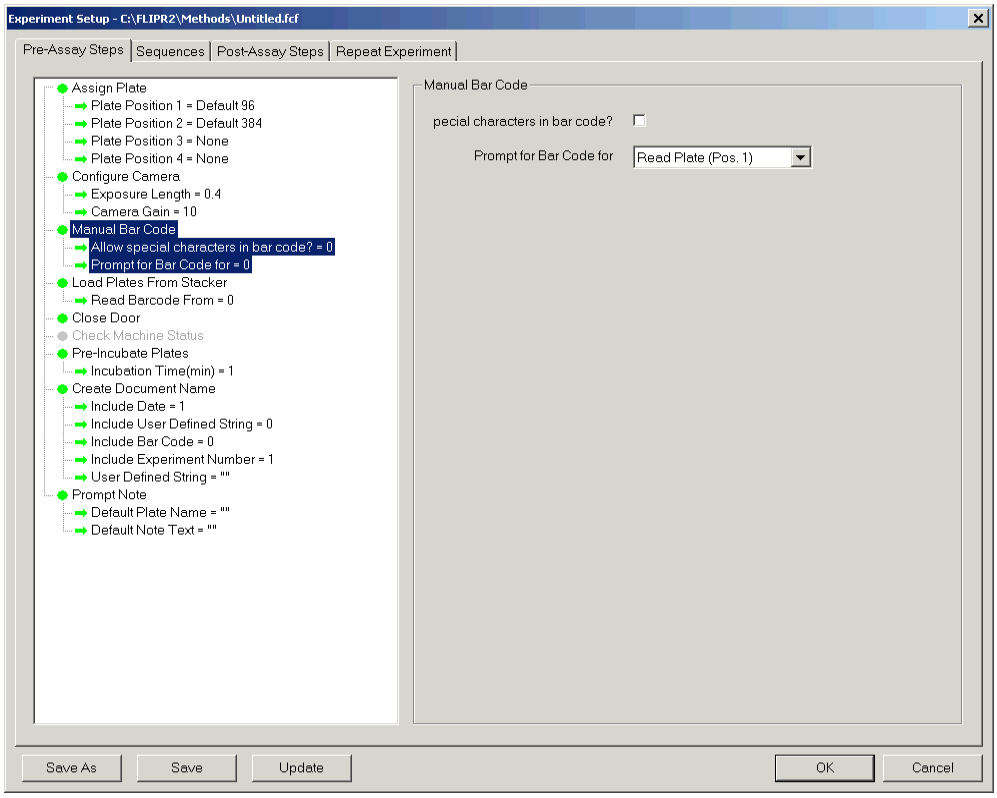


Manual Bar Code

The **Manual Bar Code** task is selected in the figure below. This feature is used when you want to enter a bar code manually *e.g.,* with a wand.

Checking the **Special Characters in bar code?** option allows you to use bar codes that contain characters that the Microsoft NT operating system doesn't allow in file names.

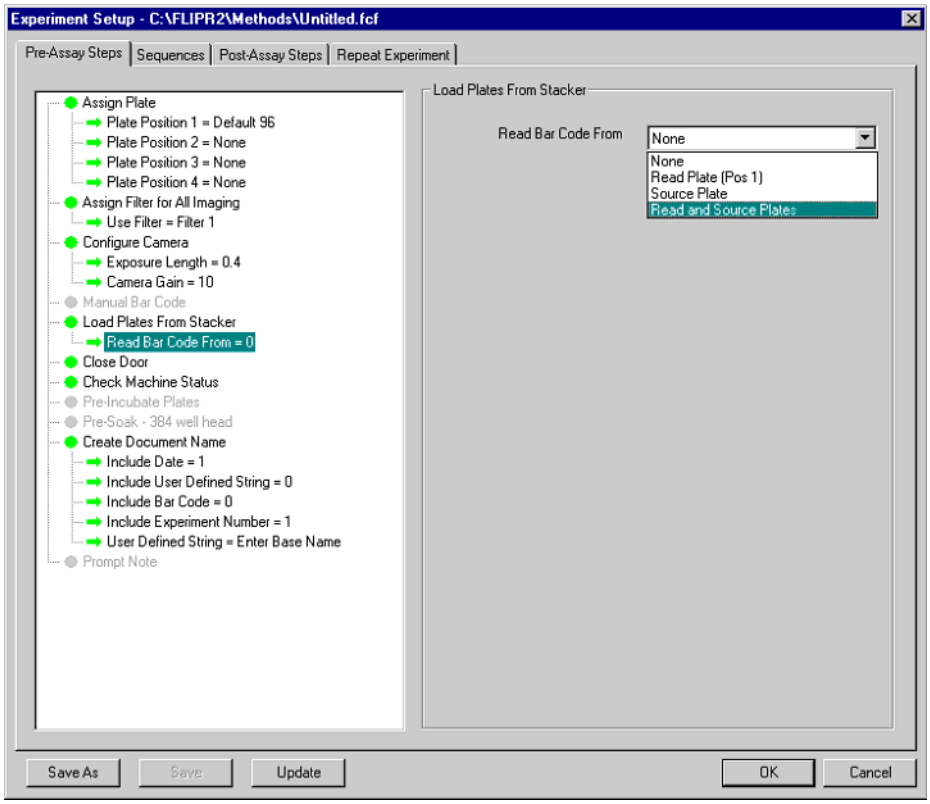
In the **Prompt for Bar Code for** box, select the appropriate plate position you want the system to pause at and wait for a bar code to be entered.



Load Plate from Stacker

This task appears only if you have a stacker and it is in use. The options are shown in the figure below.

From the **Read Barcode from** drop-down menu, choose the plate that has the bar code.



Close Door

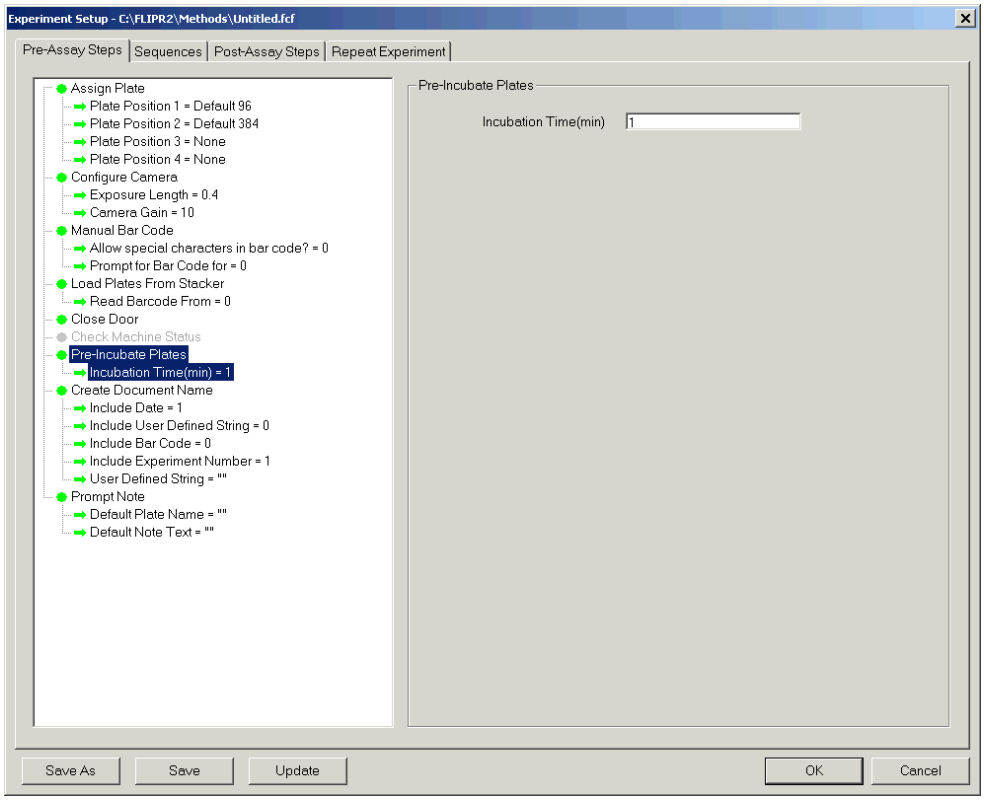
This is a required task and there are no options associated with this task. When the experiment is started, the door will close prior to executing the remainder of the tasks in this tab

Check Instrument Status

This is a required task and no options are associated with it. After the door closes, the system will check the state of hardware components that are being used for the experiment. If there is a problem, a warning dialog box will appear specifying the problem.

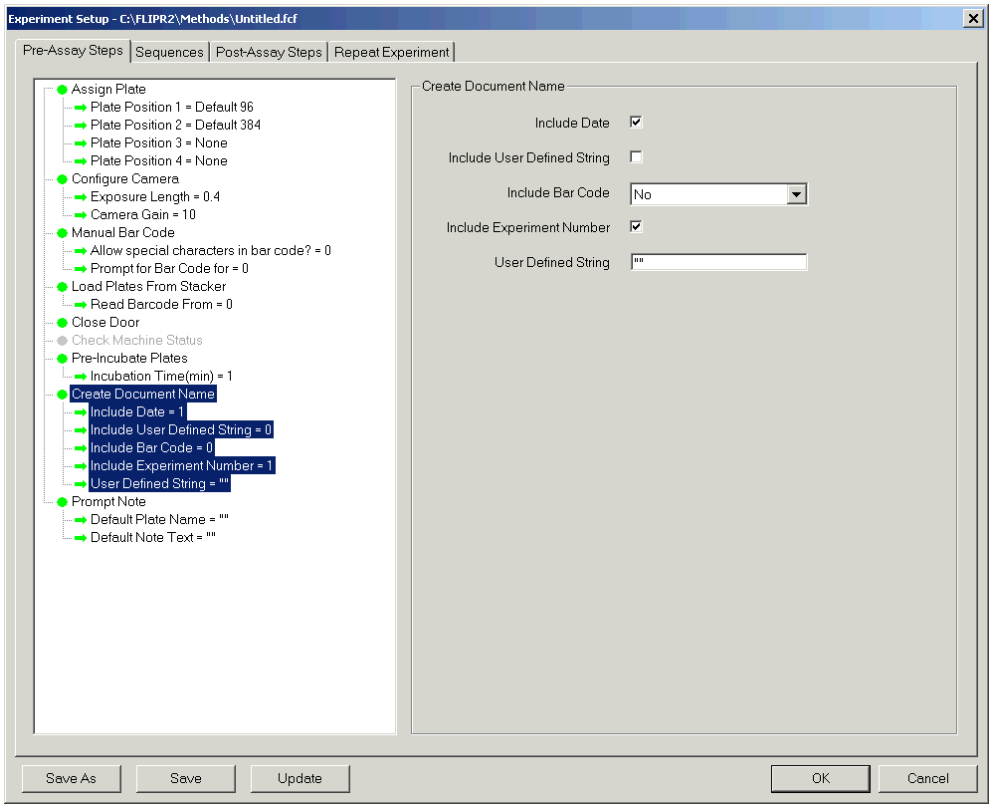
Preincubate Plate

This feature is selected in the figure below. It is used if you want to incubate the plate prior to adding a reagent or taking images. Enter the time (in min) in the **Incubation Time** field.



Create Document Name

The **Create Document Name** task is selected in the figure below. The options associated with this task are shown in the **Create Document Name** panel. These options will determine what will be included in the name of the data file created for the experiment. The order listed below is the order in which the items will appear in the file name with each item separated by an underscore.



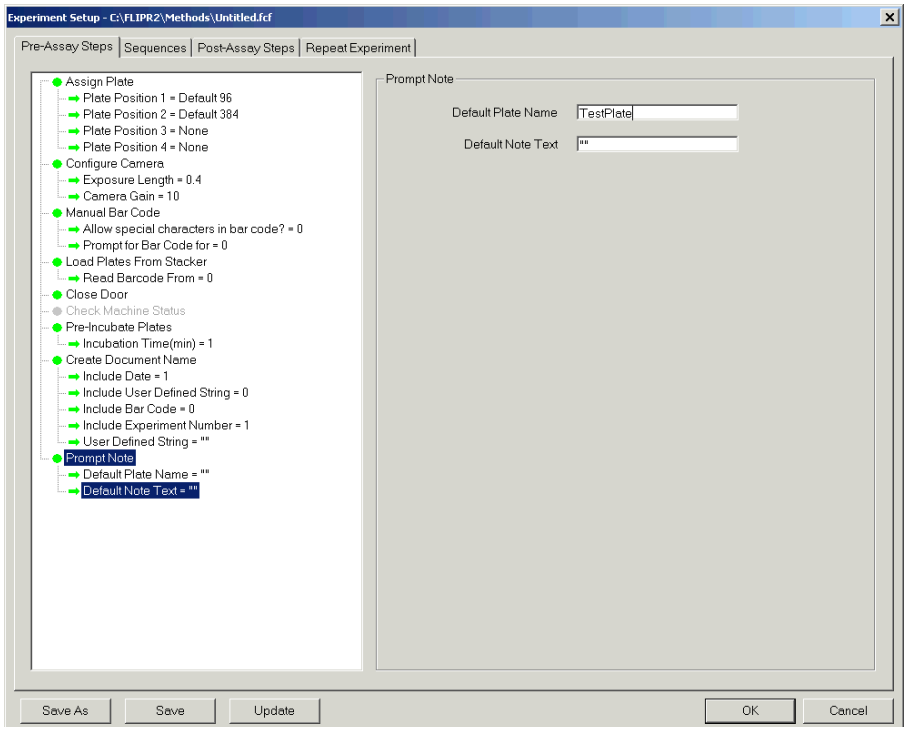
Option/Setting	Description
Include Date	When enabled, this option will include the date in the file name. **IMPORTANT** We recommend that you leave this checked. If you uncheck this option, file names that are otherwise identical will overwrite each other.
Include User Defined String	When enabled, this option allows you to enter a string of characters that will be added to the file name.
Include Bar Code	If using a barcode, select the appropriate bar code from the drop-down menu. The barcode will appear in the data file name. **Note** If the system is unable to read the bar code, it will display **Bad_bar_code_entered**.

Option/Setting	Description
Include Experiment Number	When enabled, the experiment number will be included in the data file name. The number will automatically be appended to reflect the number of runs performed with each name. **IMPORTANT** We recommend that you leave this checked. If you uncheck this option, file names that are otherwise identical will overwrite each other.
User Defined String	If **Include User Defined String** is checked, text entered in this field will be included in the data file name.

Prompt Notes

This option is linked to the **Notes Editor** dialog box. Text entered in the **Default Plate Name** and **Default Note Text** fields will appear in the **Notes Editor** dialog box.

When this option is checked, the **Notes Editor** dialog box will appear immediately after the control file is created.



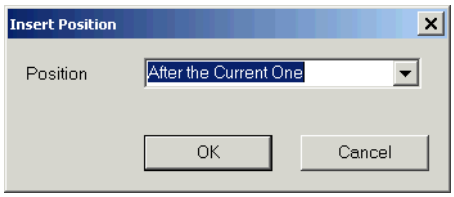
Sequences Tab

On this page of the **Experiment Setup** dialog box, you program the software to execute reagent additions, incubations, and imaging. A sequence generally consists of one set of pipettor steps and one or two imaging series. Repeat or additional reagent additions require programming another sequence. The software will execute up to 99 consecutive sequences.

Click on a sequence in the list to select it. The left and right arrow buttons above the listed sequences will move the display to previous or subsequent sequences, if there are any.

Add Sequence/Remove Sequence Dialog Box

Clicking on **Add Sequence** opens the **Insert Position** dialog box, shown below. This command is used to add a sequence. From the drop-down menu next to the **Position** field, choose the position you want to add the new sequence and then click **OK**.



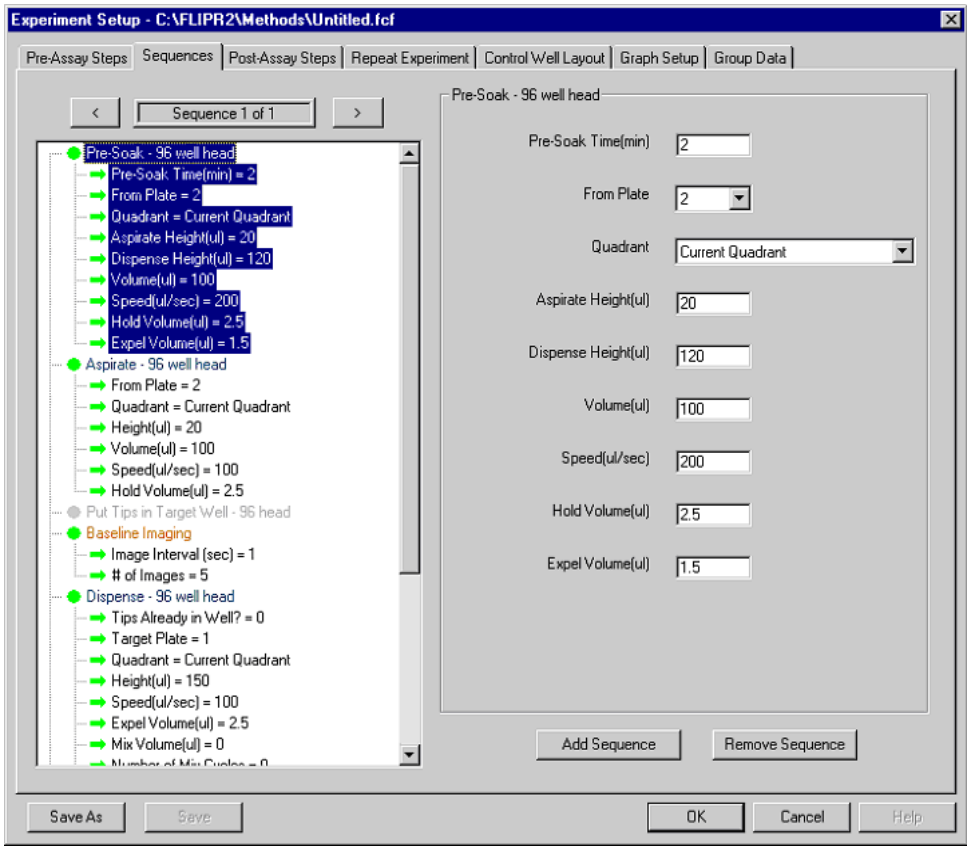
If you have more than one sequence, use the left- and right-arrows at the top of the sequence tab to move through the sequences.

To remove a sequence, display the sequence that you want to delete and then click **Remove Sequence**.

Note Sequence 1 cannot be removed, but the commands can be turned off.

Pre-Soak Tips

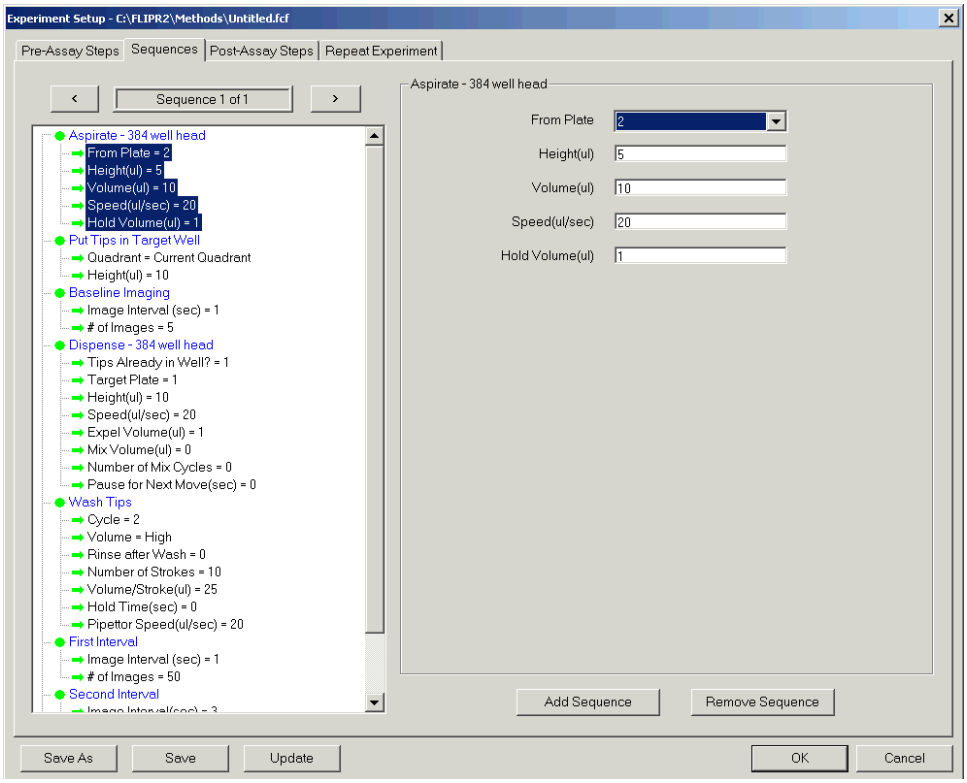
This feature is selected in the figure below. It is used if you want to expose the tips to a reagent before they aspirate and dispense it. This is recommended if the reagent is viscous or sticks easily to the tip surfaces.



Option/Setting	Description
Pre-Soak Time (min)	Enter the amount of time (in min) the tips should hold the reagent in the presoak period.
From Plate	From the drop-down menu, select the plate that has the reagent you want to use to presoak the tips.
Aspirate Height (µL)	Enter how high (in µL) from the bottom of the well the tips should start aspirating from.
Dispense Height (µL)	Enter the distance (in µL) from the bottom of the well the tips should be inserted prior to dispensing.
Volume (µL)	Enter the volume (in µL) the tips should aspirate.
Speed (µL/sec)	Enter the speed (in µL/sec) at which the tips should dispense the fluid.
Hold Volume (µL)	Enter how much of an air gap to aspirate (in µL) following the volume aspiration. An air gap is introduced to prevent fluid from leaking out of the tips before dispensing.
Expel Volume (µL)	Enter any additional volume (in µL) over the aspirate volume that you want the pipettor to dispense. Usually this number is equal to the **Hold Volume** and is equivalent to the air gap.

Aspirate

This command is used to load the tips prior to a fluid dispense or addition to the cell plate. The **Aspirate** task is selected in the figure below and shows the options associated with it.



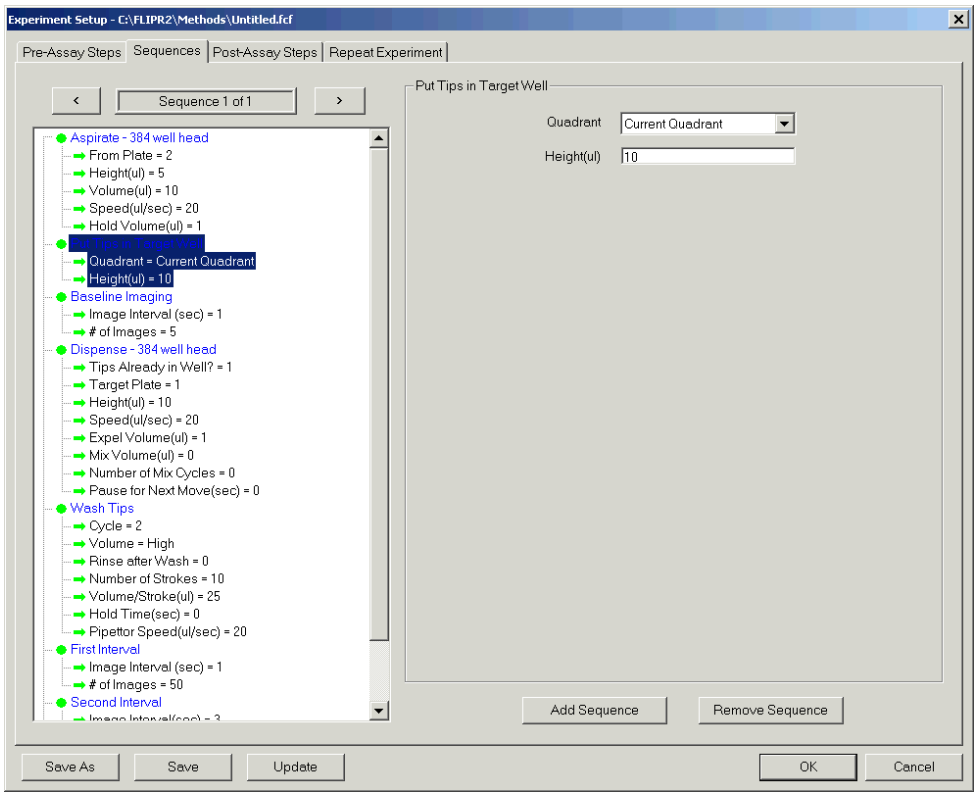
Option	Description
From Plate	From the drop-down menu, select the plate that you want the aliquot of fluid removed from. This is usually the plate that contains a compound agonist or antagonist.
Height	Defines how high from the bottom of the well (in µL) the tips should start aspirating from.
Volume	Defines how much (in µL) fluid to aspirate from the wells.
Speed	Defines the speed at which to aspirate (µL/sec)
Hold Volume	Defines how much of an air gap to aspirate (in µL) following the defined volume aspiration. An air gap is introduced to prevent fluid from leaking out of the tip before dispensing.

Put Tips in Target Well

Use this option if you want to place tips in the wells of the read plate prior to performing baseline imaging. It is suggested that you place tips above the meniscus but below the final fluid volume after addition. Placing the tips below the fluid surface can help prevent excessive turbulence that may be seen while using sensitive cell lines.

Note The height specified in **Dispense** is not used if the **Put Tips in Target Well** is activated. To avoid errors, it is recommend that you make the heights in both **Put Tips in Target Well** and **Dispense** identical.

In the **Quadrant** field, select which quadrant to place the tips in. This is meant for use when you have a 384-well cell plate and a 96-tip pipettor head.



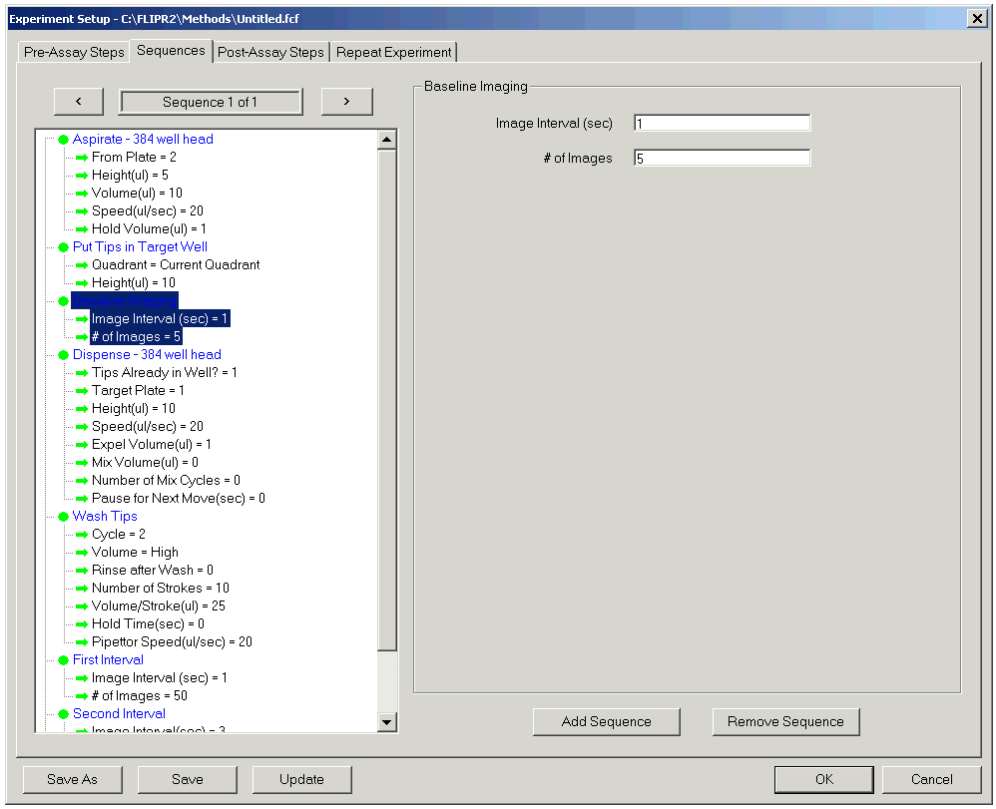
Baseline Imaging

This command is used for taking images of the cell plate before any fluid additions in order to establish a baseline.

Enter the interval between images (in sec) in the **Image Interval (sec)** field.

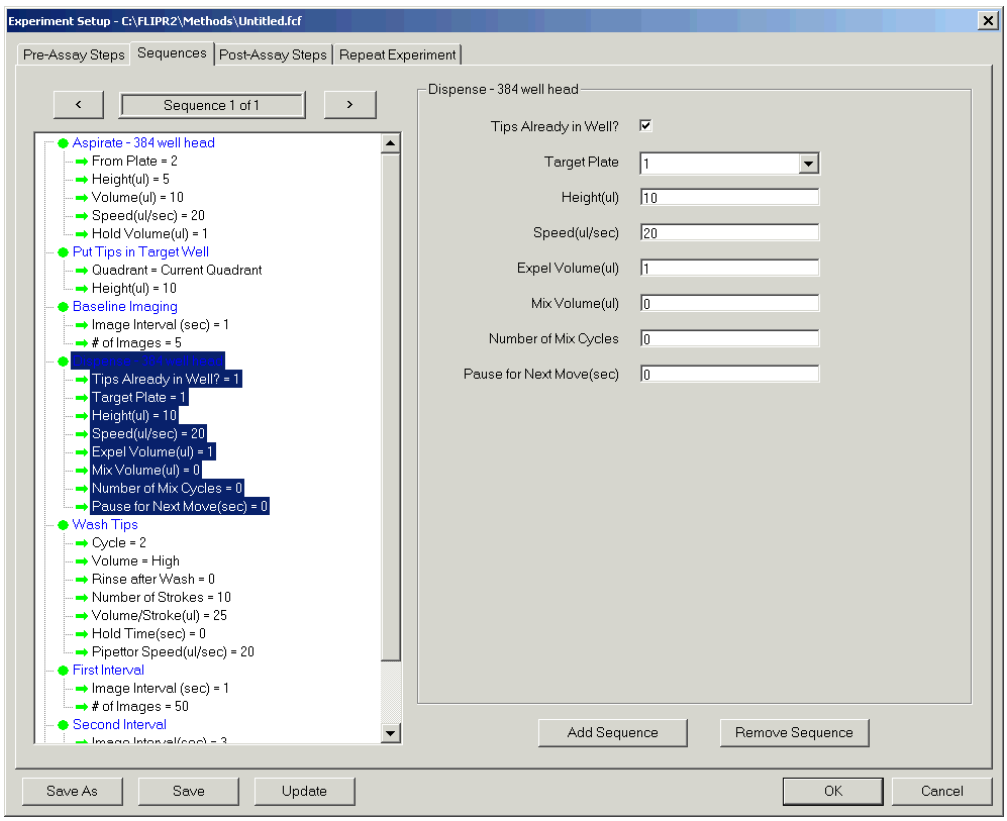
Enter the total number of baseline images you want to take in the **# of Images** field.

Note All actions programmed prior to **Baseline Imaging** will be completed in the order listed. Actions programmed after baseline imaging *e.g.*, dispensing and imaging, will occur in parallel with each other until another baseline image command is encountered. If **Baseline Imaging** is not included, the system will go to the next dispense step before imaging in the previous sequence is complete.



Dispense 384-Well Head (or 96-Well, if Using)

These settings are used to define the dispense step in the experiment sequence.



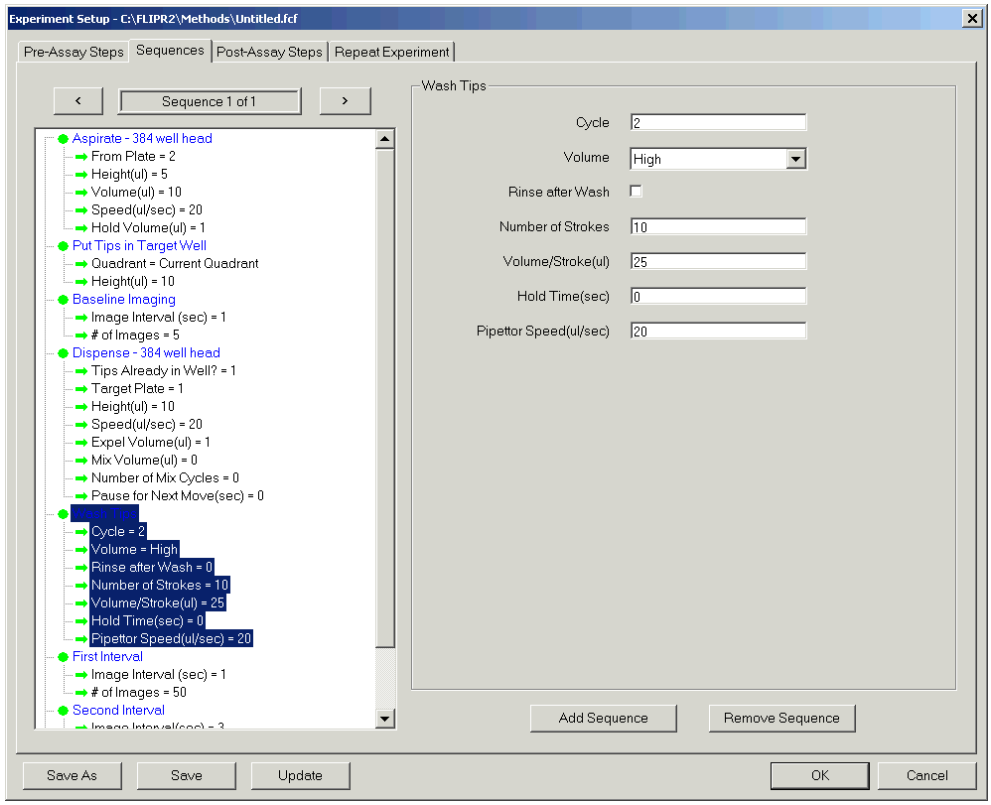
Option/Setting	Description
Tips Already in Well	Check this box if the **Put Tips in Target Well** command was implemented.
Target Plate	From the available plates, select the plate to receive the fluid dispense.
Height (µL)	Enter the distance (in µL) from the bottom of the well where the tips should be inserted prior to dispensing.
Speed (µL/sec)	Enter the speed (in µL/sec) at which the tips should dispense the fluid. The speed ranges are 1–20 µL/sec for a 384-tip pipettor and 10–100 µL/sec for a 96-tip pipettor. The ideal dispense speed takes into account the volume to be added, how fast the signal increases in response to the compounds, and the strength of the attachment of the cells at the bottom of the plate. Typical speeds for strongly adherent cells are 20 µL/sec for 384-well plates and 50 µL/sec for 96-well plates. For weakly adherent cells, the typical speeds are 5 µL/sec for 384-well plates and 10 µL/sec for 96-well plates.
Expel Volume (µL)	Enter any additional volume (in µL) over the aspirate volume that you want the pipettor to dispense. Usually this number is the same as entered in the **Hold Volume** and is equivalent to the air gap.

Option/Setting	Description
Mix Volume (µL)	Enter the volume (in µL) to be aspirated and dispensed for mixing.
Number of Cycles	Enter the number of times to aspirate and dispense the **Mix Volume**.
Pause for Next Move (sec)	Enter the time (in sec) to pause after dispensing and mixing before going to the next pipettor move. Since the imaging will be occurring at this step, it is often desirable to pause to prevent disturbances in the imaging process.

Wash Tips

This command is only available when you have a tip washer. The commands for this option vary depending on whether you are using a FLIPR^2 or FLIPR^3 system.

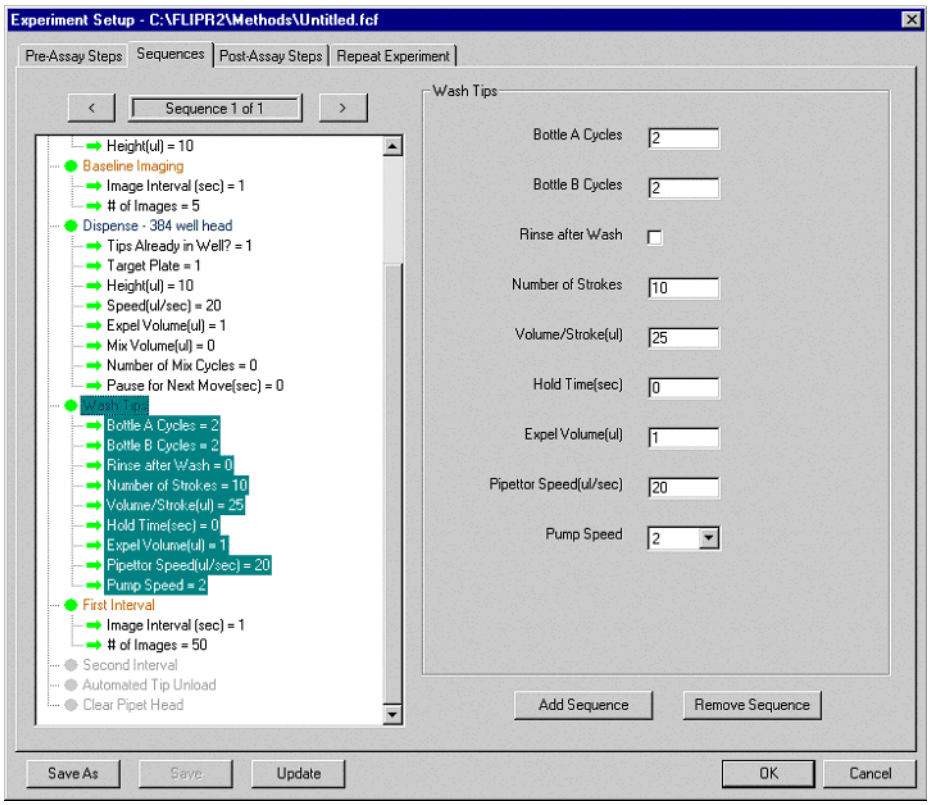
Wash Tips for FLIPR^2



Option/Setting	Description
Cycle	Enter the number of times to repeat the entire wash tip sequence.
Volume	Defines how much liquid to put in the reservoir. Select **High** or **Low**. Low is ~2/3 of the high volume.
Rinse after Wash	Check this option to rinse the tips after the wash tip cycle.
Number of Strokes	Enter the number of times to aspirate and dispense wash fluid.

Option/Setting	Description
Volume/Stroke (µL)	Enter the volume of wash fluid (in µL) to aspirate and dispense.
Hold TIme (sec)	Enter the amount of time (in sec) to wait between aspirating and dispensing.
Pipettor Speed (µL/sec)	Enter the rate (µL/sec) at which the pipettor should aspirate and dispense wash fluid.

Wash Tips for FLIPR^3



Parameter	Description
Bottle A Cycles	Enter the number of times you want to run the wash cycle using solution from bottle A. For each cycle, the pipettor executes the value in the **Number of Strokes** field.
Bottle B Cycles	Enter the number of times you want to run the wash cycle using solution from bottle B. For each cycle, the pipettor executes the value in the **Number of Strokes** field. **Note** **Bottle A** cycles will be executed before **Bottle B** cycles.

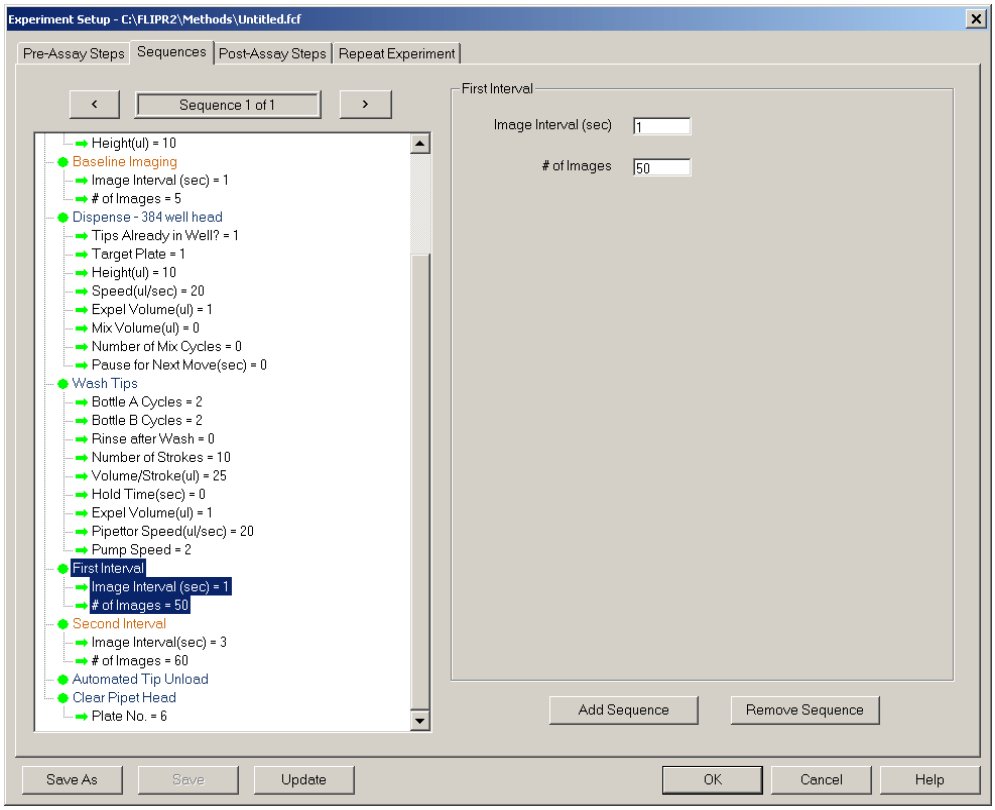
Parameter	Description
Rinse after wash	Check this option if you want the wash basin rinsed once after completing bottle A and B cycles. **Note** If you are using both bottles, there will be a rinse after the last cycle of bottle A before starting bottle B and again after completion of the last cycle of bottle B.
Number of Strokes	Enter the number of times that you want the pipettor to aspirate and expel wash solution from the tips.
Volume/Stroke (µL)	Enter the volume of wash solution the pipettor should aspirate and expel for each stroke.
Hold Time (sec)	Enter the amount of time to wait between aspirating and expelling the fluid.
Expel Volume (µL)	Enter the expel volume for each stroke. **Note** This value is typically greater than the stroke volume to ensure fluid is completely expelled.
Pipettor Speed (µL/sec)	Enter the speed the pipettor should aspirate and expel fluid.
Pump Speed	Enter the speed (1(slow) or 2(fast)) you want the pump to fill and empty the wash reservoir

First Interval/Second Interval

First Interval and **Second Interval** tasks refer to the imaging steps of the experimental sequence. For each series of images, you can have only one time interval between images.

The first interval will be the images taken immediately upon and shortly after fluid addition. Since this is usually the most rapid phase of the response, the time interval between images for the first interval is usually very short and the number of images is larger than for the second interval.

The second interval will document the latter part of the response which is diminishing in intensity and subject to less rapid changes in fluorescence. For this reason, the time interval for the second response is usually longer and the number of images taken less than for the first interval. The **First Interval** task is selected in the figure below. The **Second Interval** task has the identical parameters as the **First Interval**.



Option/Setting	Description
Image Interval (sec)	Enter the time (in sec) between images. **Note** The time should be no less than the exposure time + the readout time (see *"Baseline Imaging"* on page 4-89). If it is less, then the experiment will run as fast as the computer will be able to support.
# of Images	Enter the total number of images for the first interval.

Automated Tip Unload

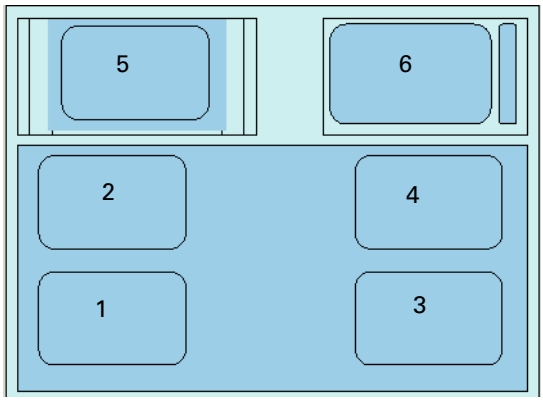
Activating this task instructs the pipet head to unload the tips after dispensing.

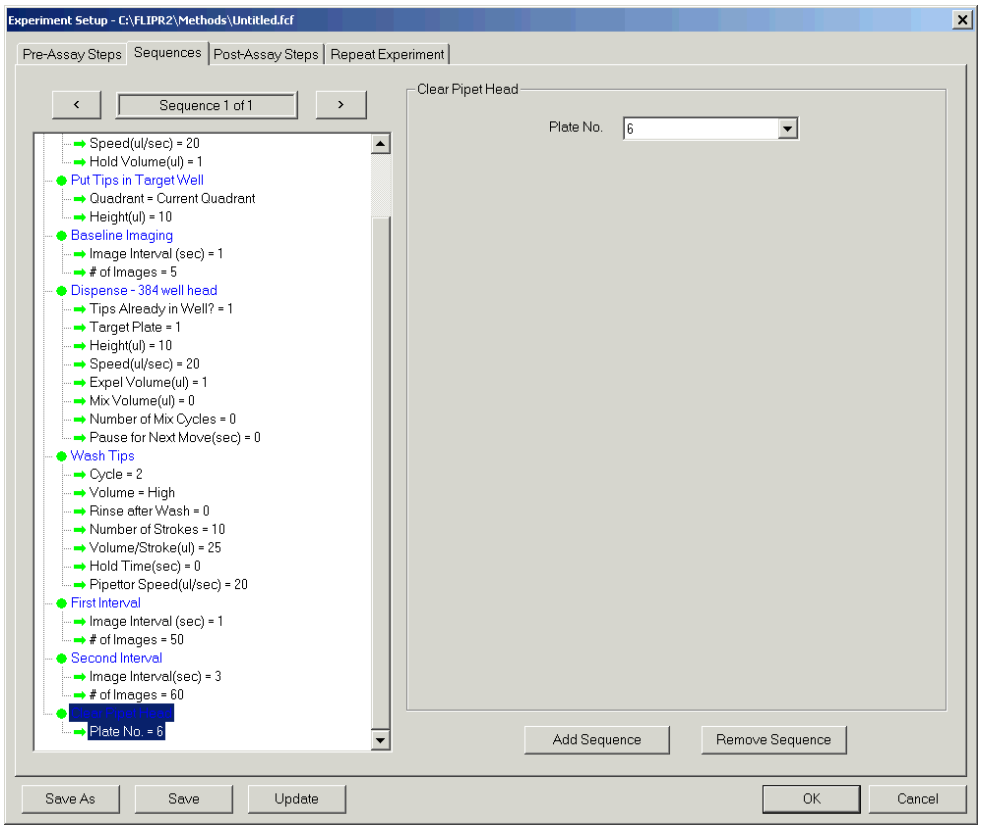
Clear Pipet Head

This task is used to move the pipettor head to one of six positions on the stage. Select a stage position (1–6) from the **Plate No.** drop-down menu.

Note Clearing the pipet head only moves the pipettor to the location indicated and does NOT empty the tips.

Plate Position





Post Assay Dialog Box

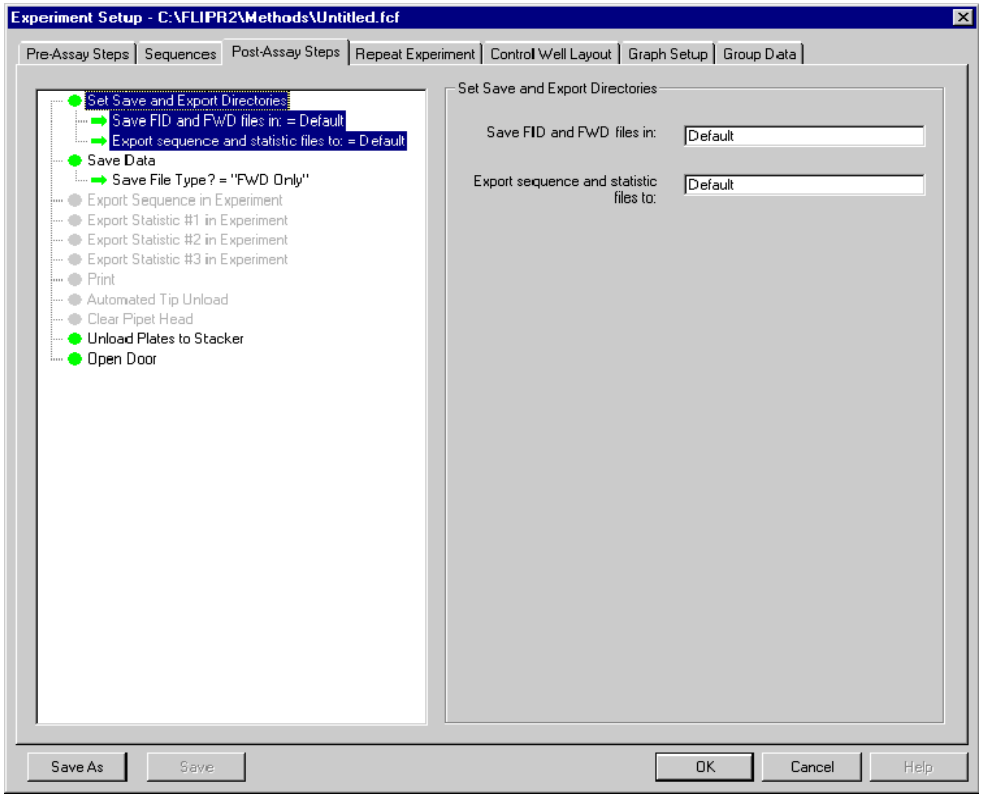
In this dialog box, you may choose data file options, grouping tasks, and exporting functions.

Save and Export Directories

Click on **Set Save and Export Directories.**

In the text fields to the right, enter the file directory location where to save the *.fid and *.fwd data files and the export sequence and statistic files.

Note These fields do not have a **Browse** feature so you must know the path to the desired folder and it must exist.



Save Data

Click on **Save Data** in the task list on the left side of the screen.

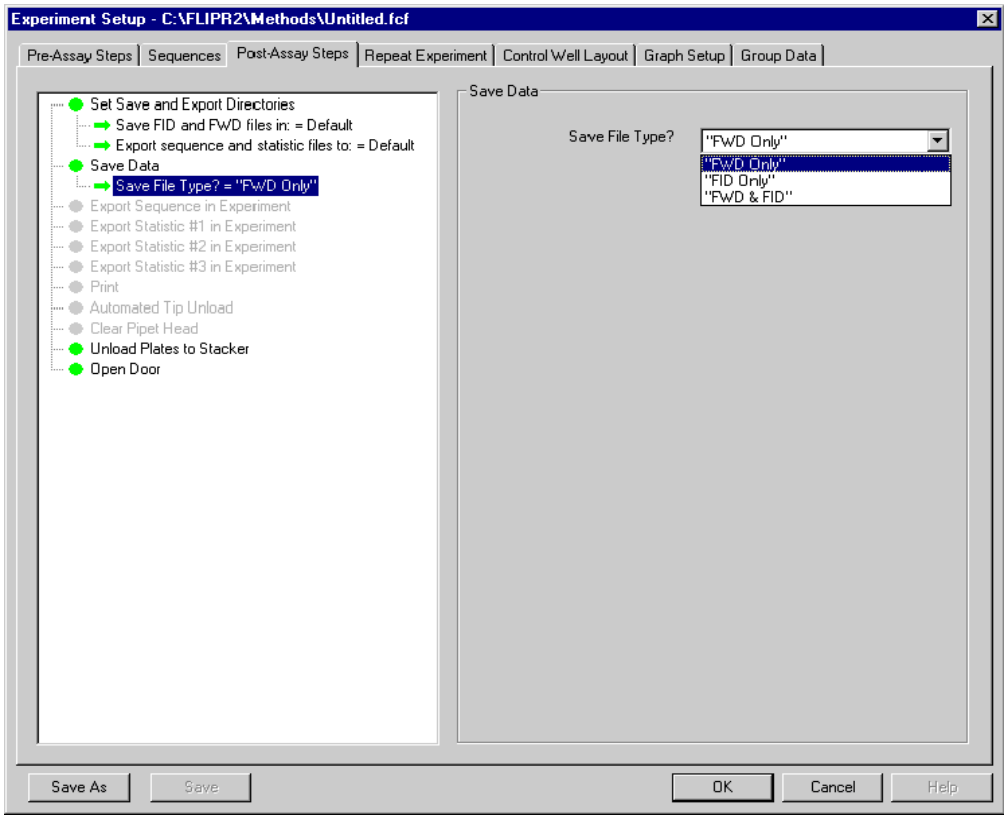
Click on the drop-down menu next to **Save File Type?** and select the desired file format.

The *.fwd format (FLIPR Well Data) file contains the signal intensities saved as count numbers and graphic plots of the experimental data. The file is typically 30–50 kilobytes in size for a 4–5 min assay. The *.fid format (FLIPR Image Data) contains the signal intensities saved as count numbers, graphic plots and all of the well images taken during the experiment. The well images can be replayed later to review abnormalities seen during the experiment. A *.fid file is typically several megabytes in size for a 4–5 min assay.

Note The *.fid files are preferred during assay development to check for dye leakage and whether cells are being dislodged from the well surface. FLIPR Image Data files require much more memory than *.fwd files. Many users will save both file types initially and then after examining the data, delete the *.fid file. *fid files can be saved as *.fwd files at a later date, but the reverse is not possible.

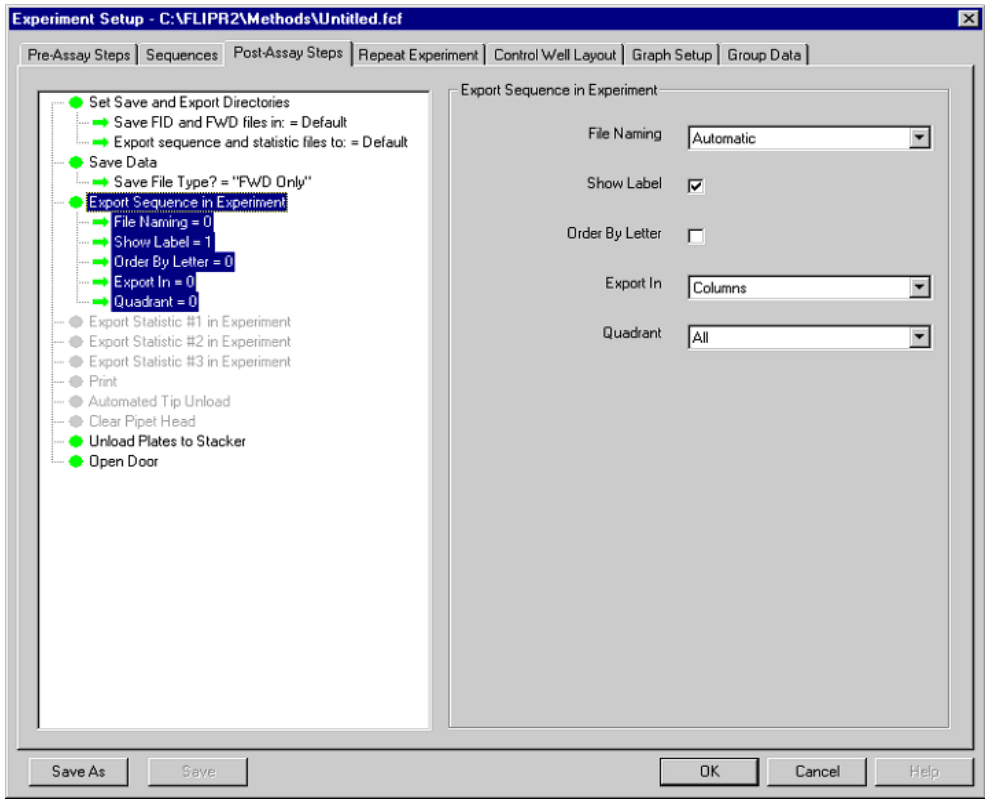
Note *.fwd files will appear as a *.fid file until the data file is closed. This occurs because the image remains in the memory after an experiment is run.

Note Clicking the **Stop** button will cause the software to immediately dump all data to the save directory. Both a *.fid and a *.fwd file will be saved during this operation regardless of what type of file is designated in the FLIPR control file.



Export Sequence in Experiment

These options allow you to determine what items to include in the naming of the exported sequence file and how to format the exported data.

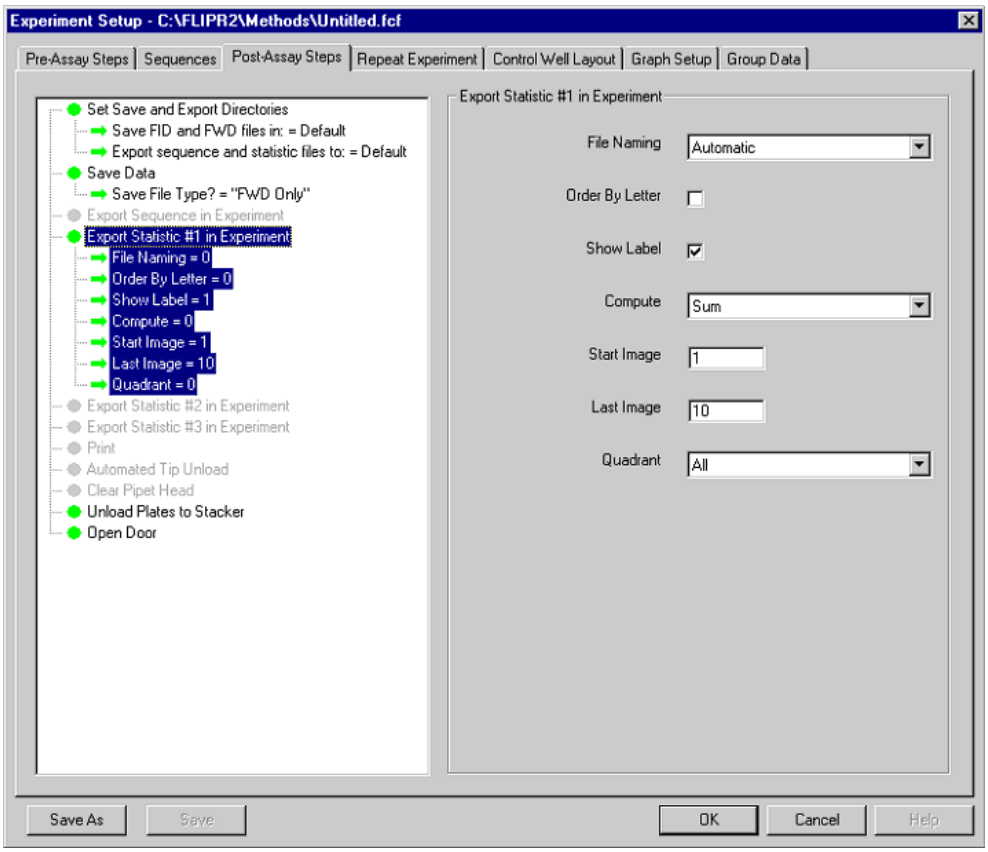


Option/Setting	Description
FIle Naming	**Automatic** will assign the date to the file. **Prompt User** will use what you defined in the pre-assay step.
Show Label	Check this option to have the resulting output file contain information about the processing options selected.
Order by Letter	Check this option if you want the columns in the exported file to be ordered A1, A2, A3; otherwise, the order will be A1, B1, C1.
Export In	Choose whether to have the data exported in columns (images/well appearing vertically) or rows (images/well appearing horizontally). **Note** If you will be importing the data to a Microsoft Excel file, there is a limitation of 256 columns. Therefore, if you are using 384-well plates, you will have to export in rows.
Quadrant	Choose whether to have the data from a single quadrant or all of the quadrants exported.

Export Statistics #1/#2/#3

The **Export Statistics** options allow you to define the naming and parameters of the exported statistics file.

A maximum of three different statistic files can be executed and saved per experiment.



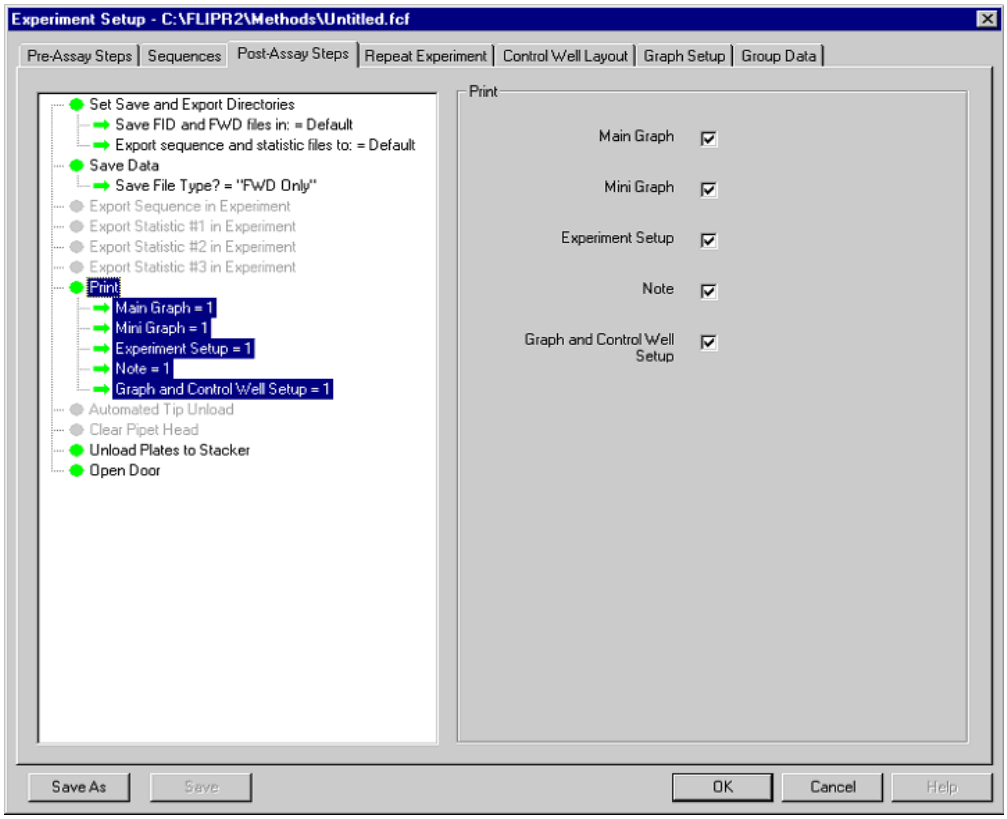
Option/Setting	Description
File Naming	Choose whether to have the statistics files named automatically or to be prompted to name each file when it is saved.
Order by Letter	Check this option if you want the columns in the exported file to be ordered A1, A2, A3; otherwise, the order will be A1, B1, C1.
Show Label	Check this option to have the resulting output file contain information about the processing options selected.
Compute	Choose a mathematical operation that you want performed on designated samples; **sum**, **average**, **max**, **min**, or **max–min**.
Start Sample	Define the first sample to be included in the computation selected in **Compute**.

Option/Setting	Description
Last Sample	Define the last sample to be included in the computation selected in **Compute**. **Note** Start may equal last but start cannot be greater than last, and both start and last must be less than or equal to the total number of images.
Quadrant	Choose one or all of the quadrants that should be included in the statistics computations.

Print

The **Print** options allow you to choose which items of the experiment to print at the end of the experiment.

Check the items that you want printed.

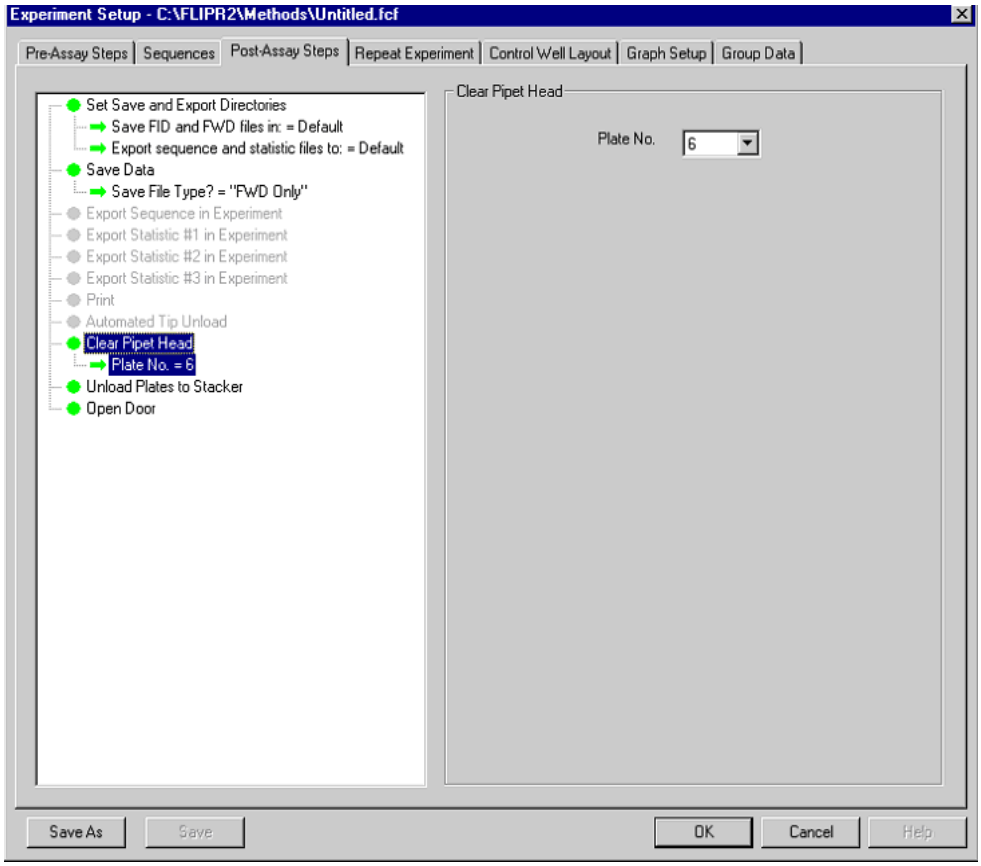


Automated Tip Unload

Activating this task instructs the pipet head to unload the tips after dispensing.

Clear Pipet Head

This task is used to move the pipettor to one of six positions on the stage after the sequence is completed. Select a stage position (1–6) from the **Plate No** drop-down menu (see *"Automated Tip Unload"* on page 4-95 for a diagram of plate positions).



Unload Plates to Stacker

This is a required task and will appear automatically in the **Post-Assay Steps** dialog box if you are using a stacker. The stacker will automatically unload after completion of the sequence.

Open Door

This is a required task and will automatically appear in the **Post-Assay Steps** dialog box. The door will automatically open after completion of the sequence.

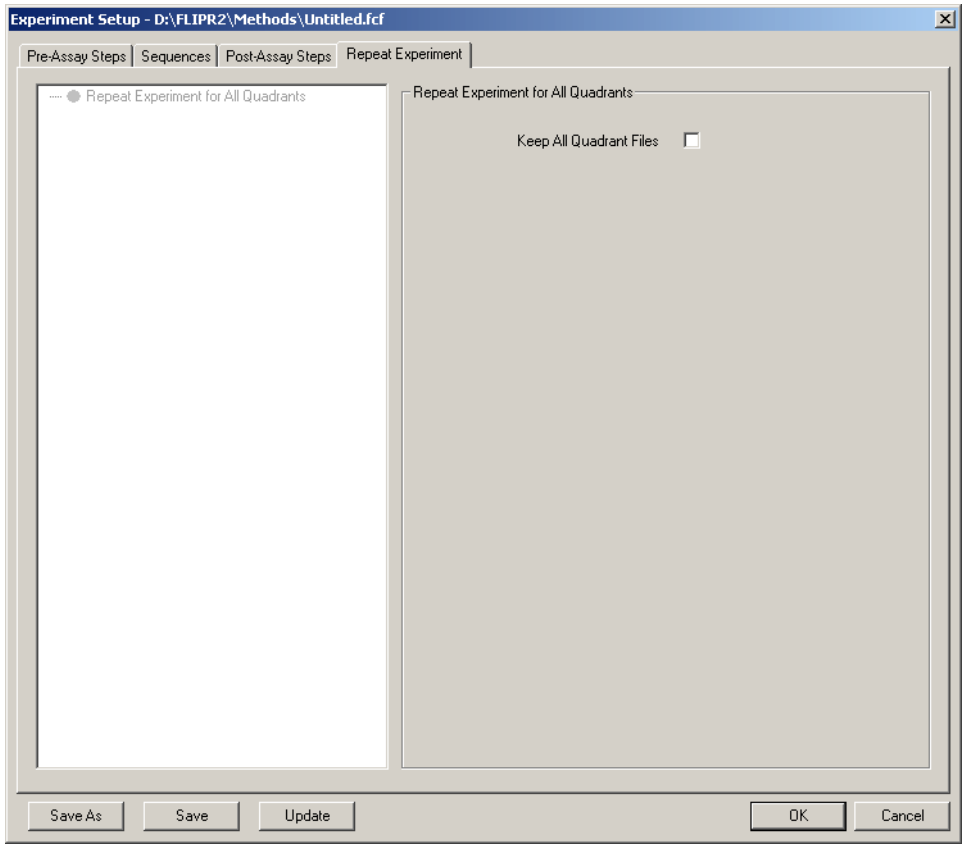
Repeat Experiment Tab

On this page of the **Experiment Setup** dialog box, you have the option of automatically repeating the experiment four times, once in each quadrant of a 384-well plate with a 96-tip pipettor. The compound plate can be a 96-well, 384-well, or boat plate.

If **Keep all Quadrant Files** is checked, then all four experiments will be automatically saved.

Note This option is only available when you are using a 96-well pipettor head in 384-well format.

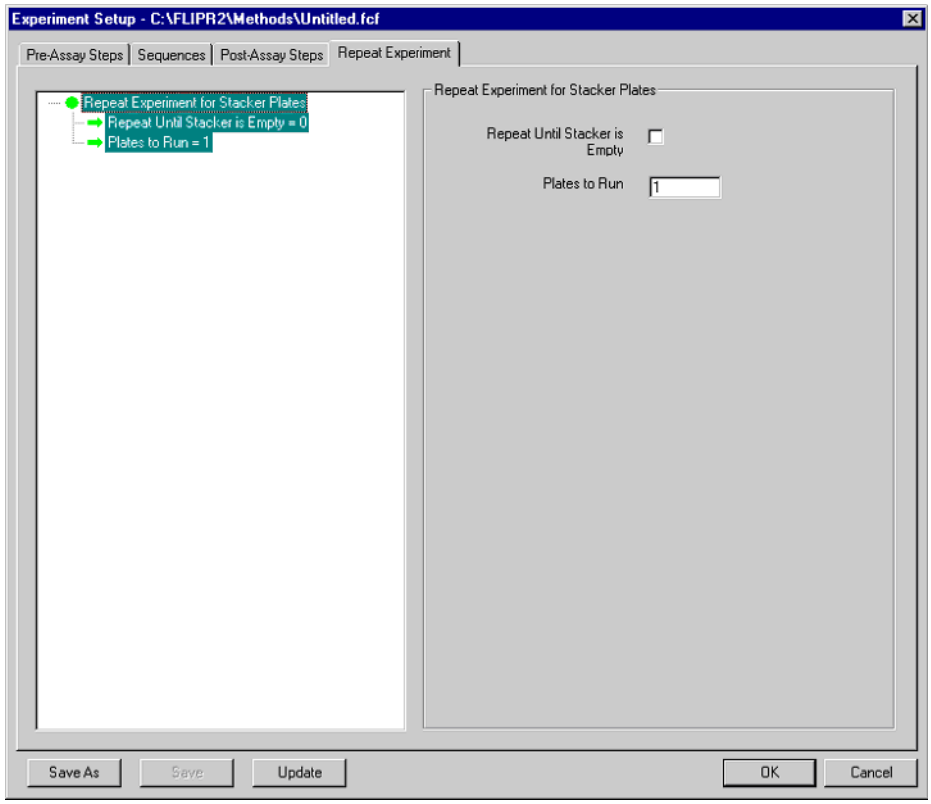
Note When using the **Repeat for All Quadrants** function in stacker mode, the FLIPR instrument will shuttle out the read plate and retrieve a new one for each quadrant. If you want to do multiple quadrants on a single read plate in stacker mode, you will need to create an additional sequence for each quadrant and deselect **Experiment for All Quadrants**.



If Using the Stacker

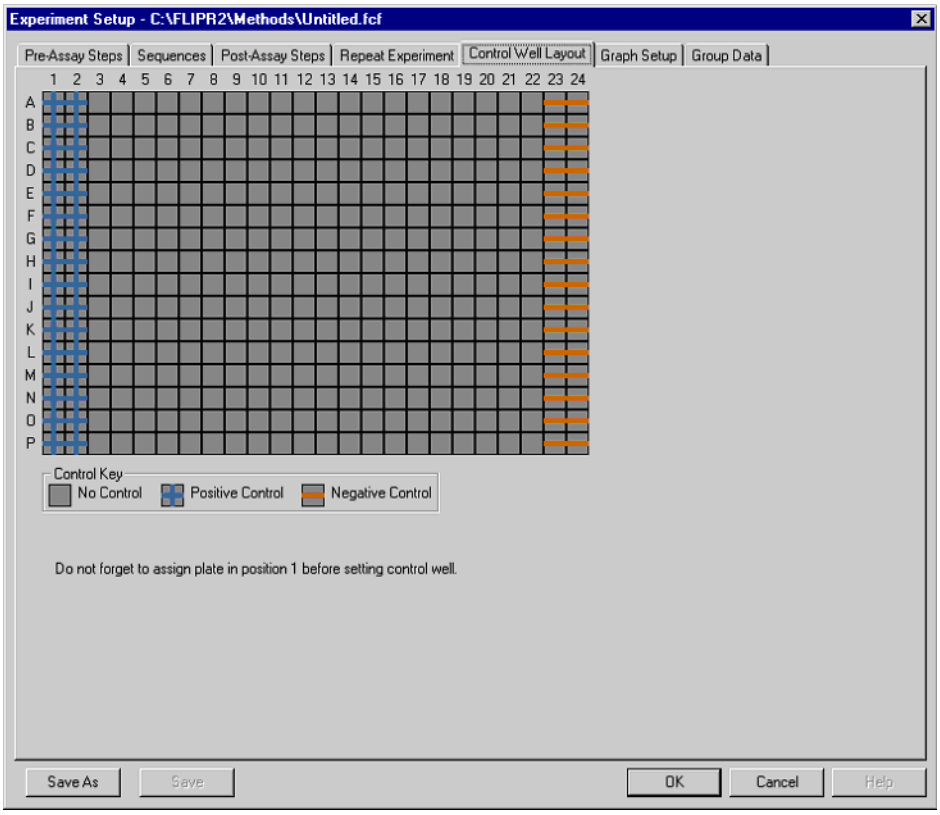
If you are using the stacker, then you will see the following options on the **Repeat Experiment** tab.

Check **Repeat Until Stacker is Empty** if you want to run all the plates in the stacker, or in the **Plates to Run** field, enter the number of plates you want to run from the stacker.



Control Well Layout Dialog Tab

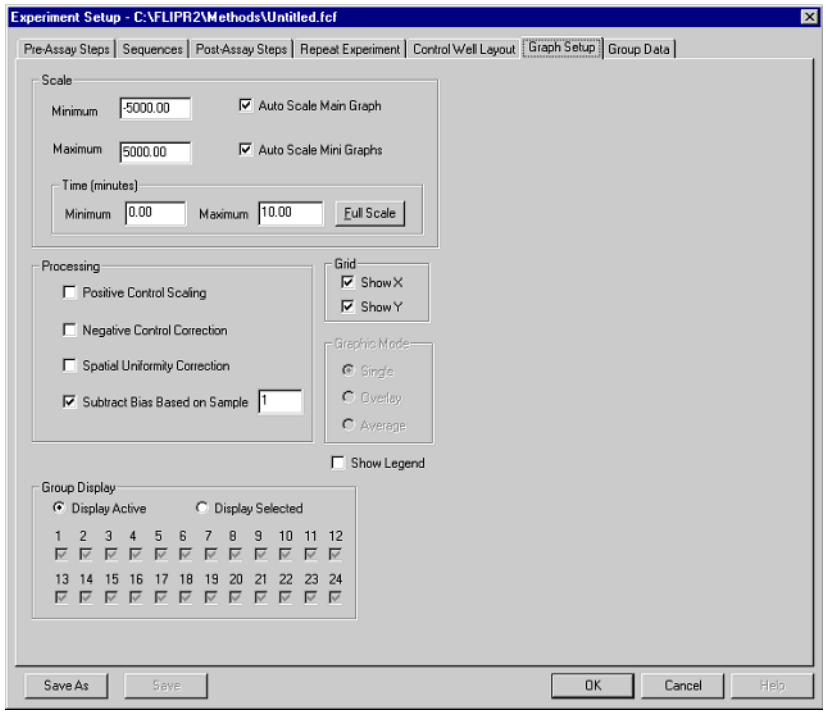
The options set in this dialog tab are used as the default when starting an assay. See *"Control Well Layout Tab"* on page 4-61 for a complete description of this dialog tab and the functions it contains.



Graph Setup Dialog Tab

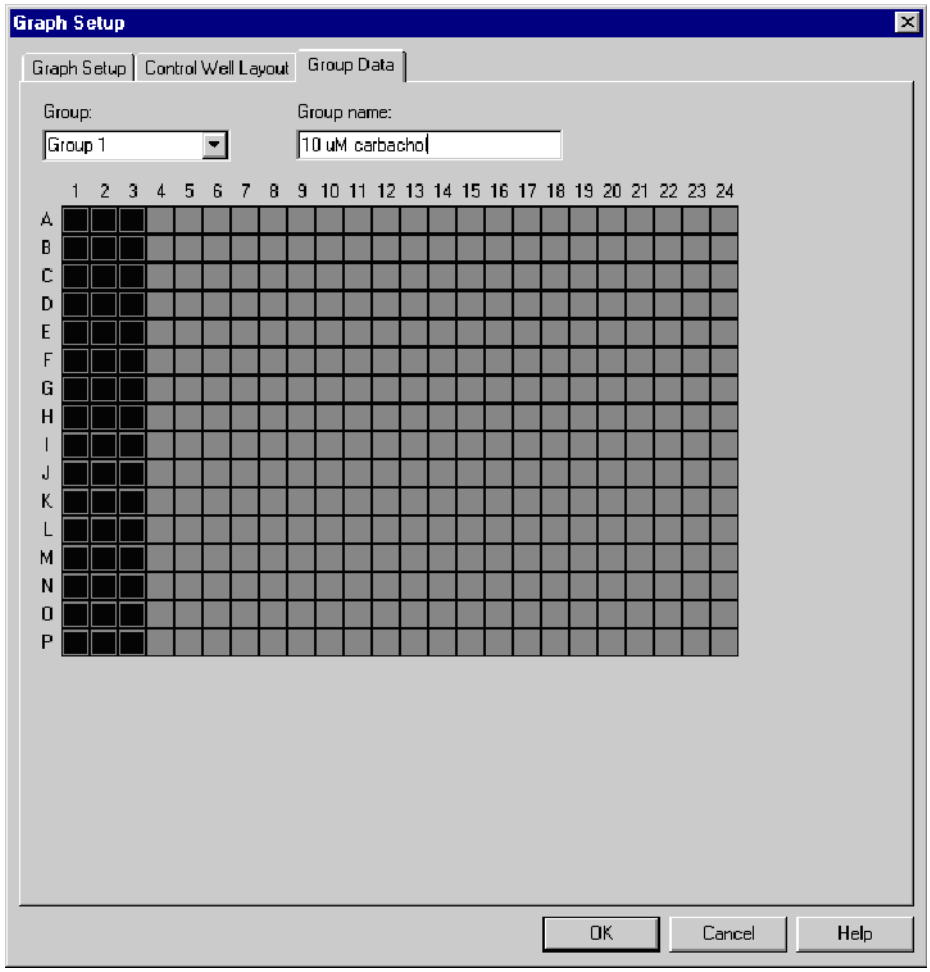
The options set in this dialog tab are used as the default when starting an assay. See *"Graph Setup Tab"* on page 4-58 for a complete description of this dialog tab and the functions it contains.

The default settings can be overridden on an individual basis without modifying the default values for the next assay by using the **Graph Setup** dialog box. This can be accessed by choosing **Setup** from the **Graphs** menu.



Group Data Dialog Tab

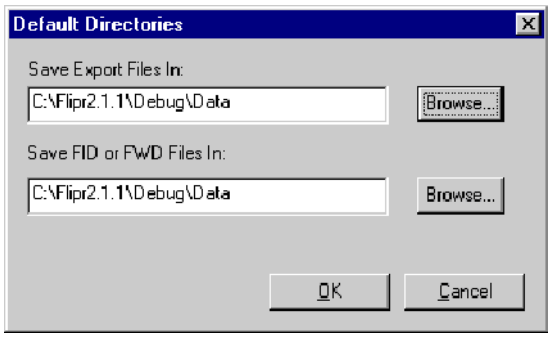
The options set in this dialog tab are used as the default when starting as assay. See *"Group Data Tab"* on page 4-62 for a complete description of this dialog tab and the functions it contains.



Setup Menu Dialog Boxes and Windows

File Directories Dialog Box

This dialog box appears when you choose **File Directories** from the **Setup** menu. The settings in this dialog box allow you to choose naming options for your data file and to specify the directory for saving them.



Item	Description
Default Directories	The default directories for saving exported files and data files are displayed when the dialog box is opened. To change the directory, click **Browse** and locate the desired directory. It is recommend that you save all *.fid and *.fwd files on the local hard drive to ensure the data is not lost if your server fails during an experiment. Files can be transferred at a later point to the appropriate drive.

Plate Library Window

About the Plate Library

This window appears when you choose **Plate Library** from the **Setup** menu. It allows you to add a plate and mask to the software.

Note In FLIPR^3 instruments, the well images that appear in this window will be rectangular or elliptical (depending on your plate type) not square or circular. This visual distortion is the result of the way the camera in FLIPR^3 instruments maps the pixels. It does not interfere with or introduce any errors to the data collection.

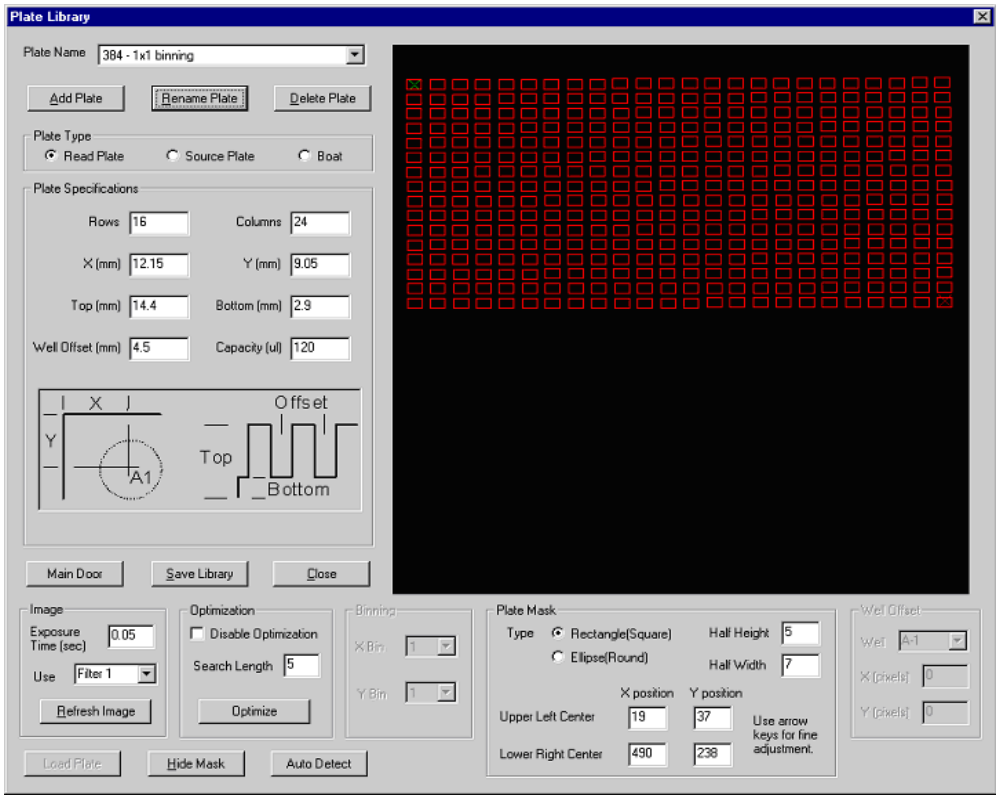
Three default plates are included with the software:

- Default 96-well plate
- Default 384-well plate
- Default 1536-well plate

Note If these default plates are inadvertently deleted, they can be re-entered. Their dimensions are provided in the table below.

Default Plate	X(min)	Y(min)	Top	Bottom	Well Offset	Capacity
96-well (Nunc)	14.4	11.3	14.6	3.7	9	370
384-well (Greiner)	12.15	9.05	14.4	2.9	4.5	120
1536-well (Greiner)	12.15	9.05	14.4	2.9	4.5	120

Plate Library Description



item	Description
Plate Name	The drop-down list next to the field lists the available plates in the system. To display the parameters of a plate, select it from the list.
Add Plate	Click **Add Plate** to add a new plate to the system. A dialog box will appear asking for a new name.
Rename	Click **Rename** to change the name the plate displayed in the **Plate Name** field.
Delete Plate	Click Delete **Plate** to delete the plate displayed in the **Plate Name** field.
Plate Type	Choose a plate type option. **Read Plate**—Refers to the plate that will be imaged **Source Plate**—Refers to a plate type that will be used only for adding reagents to the imaged plate. **Boat**—Refers to a plate type that is a large volume container that will be used to add a single reagent to the imaged plate. **Note** When **Source Plate** or **Boat** plate types are selected, the imaging part of the plate library setup will not be available.

item	Description
Plate Specifications	This panel requires the entry of the physical dimensions of the plate (refer to the diagram in the lower area of the box). **Note** For best results, it is recommended that you get plate specifications from the plate manufacturer. **Rows**—Number of rows in the plate **Columns**—Number of columns in the plate **X (min)**—Distance (in mm) from the side of the plate to the center of well A1. **Y (min)**—Distance (in mm) from the top of the plate to the center of well A1. **Top**—Distance (in mm) from the bottom of the plate to the top of the well. **Bottom**—Distance (in mm) from the bottom of the plate to the bottom of the well. **Well Offset**—Distance (in mm) from the center of one well to the center of the adjacent well. **Capacity**—Maximum volume (in µL) the well can hold.
Main Door	This button acts like a toggle to open and close the main instrument door.
Save Library	Click **Save Library** to save the plate and all of the settings in the software. This plate will now be available in the **Experiment Setup** dialog box.
Close	Click **Close** to close the **Plate Library** window.
Image	This panel allows you to change the exposure time for taking an image. **Exposure**—Enter the length of time (in sec) for the exposure **Use**—Allows you to change filters for taking an image. Select the filter from the drop-down menu. **Refresh Image**—Causes the camera to take and display a new image with the current settings.
Optimization	This panel allows you to control the optimization feature for defining a mask. The optimization process chooses the best fit mask given the parameters entered in the **Plate Library** dialog box. **Disable Optimization**—Check this box to turn off the optimization feature. If this box is not checked, the optimization will always be carried out. Optimization aligns a box around the brightest portion of each well. **Search Length**—Enter the distance (in pixels) from each well that you want the optimization algorithm to consider when executing the optimization. **Optimize** —Click this button to apply the optimization settings.

item	Description
Binning	Grouping of pixels in the X or Y direction *e.g.*, a binning of 2 X 2 means that every 4 pixels will be represented by one pixel. **Note** This feature is disabled in normal operating mode.
Plate Mask	This panel allows you to align the mask more closely to the plate, if necessary. **Type**—Select the correct shape of wells in the plate. **Half Height**—Vertical dimension of the mask for a single well (in pixels). **Half-Width**—Horizontal dimension of the mask for a single well (in pixels). **Upper Left Corner**—Click in the center of the upper-left well (A1). The X and Y values will be entered automatically. **Lower Right Corner**—Click in the center of the lower-right well. The X and Y values will be entered automatically.
Well Offset	This option allows you to alter the theoretical center of the mask calculated by the **Auto Detect** function. It allows you to move the mask for a well in the X and/or the Y direction (in pixels). Choose the well you want to offset from the drop-down menu and then enter a value in the X or Y fields and press the **Enter** key. The mask will update to reflect the changes. **Note** This feature is disabled in normal operating mode.
Load Plate	Click **Load Plate** to move an inserted plate into the read position.
Hide Mask/Show Mask	Click **Hide Mask** to remove the mask (red outlines) from the image. When the mask is hidden, the button will display **Show Mask**. Click **Show Mask** to re-display the mask.
Auto Detect	Click **Auto Detect** to cause the software to examine the image and display the best-fit mask.

Adding a Read Plate to the Software

Use the description of the **Plate Library** above as a reference guide for the options and parameters available when adding a plate to the software.

To add a plate and mask to the software:

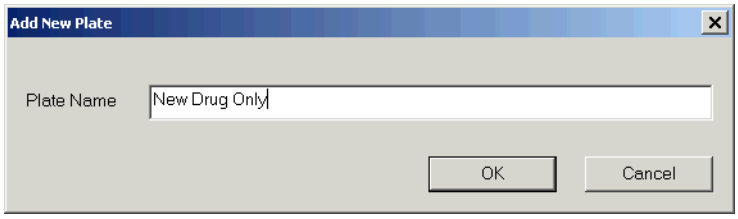
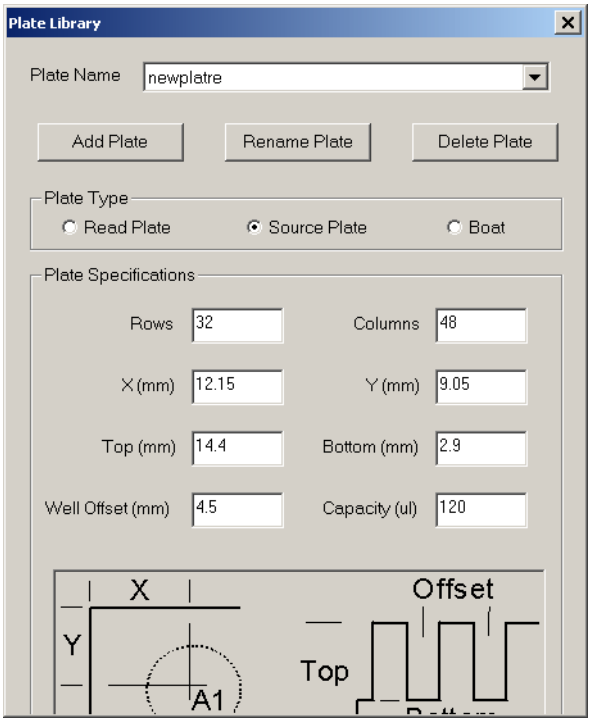
Step	Action
1	Prepare a plate of the type you want to add that has uniform luminescence or fluorescence in every well.
2	Place the plate on **Position 1** on the stage and close the door to the instrument.
3	Choose **Plate Library** from the **Setup** menu.
4	Click **Add Plate**, enter the name of the new plate in the dialog box and click **OK**. The new plate name should appear in the **Plate Name** field.

To add a plate and mask to the software: *(continued)*

Step	Action
5	Select **Read Plate** in the **Plate Type** panel.
6	Enter the plate dimensions in the **Plate Specifications** panel.
7	Click **Auto Detect**.
8	Adjust the **Plate Mask** values to center the mask over the image, if necessary.

Adding a Source or Boat Plate

To add a source or boat plate:

Step	Action
1	Choose **Plate Library** from the **Setup** menu.
2	Click **Add Plate** and enter the name of the new plate in the dialog box and click **OK**. The new plate name should appear in the **Plate Name** field. 
3	Select **Read Plate Source Plate** or **Boat** in the **Plate Type** panel. **Note** If **Source Plate** or **Boat** is selected, only the **Plate Specifications** options will be available. 

To add a source or boat plate: *(continued)*

Step	Action
4	Enter the number of **Rows** and **Columns** in the **Number of Wells** panel.
5	Enter the dimensions in the **Dimensions** panel.
6	Click **Save Library**. Close the window by clicking the **X** in the upper-right corner or by clicking the **Close** button.

Calibration Dialog Box

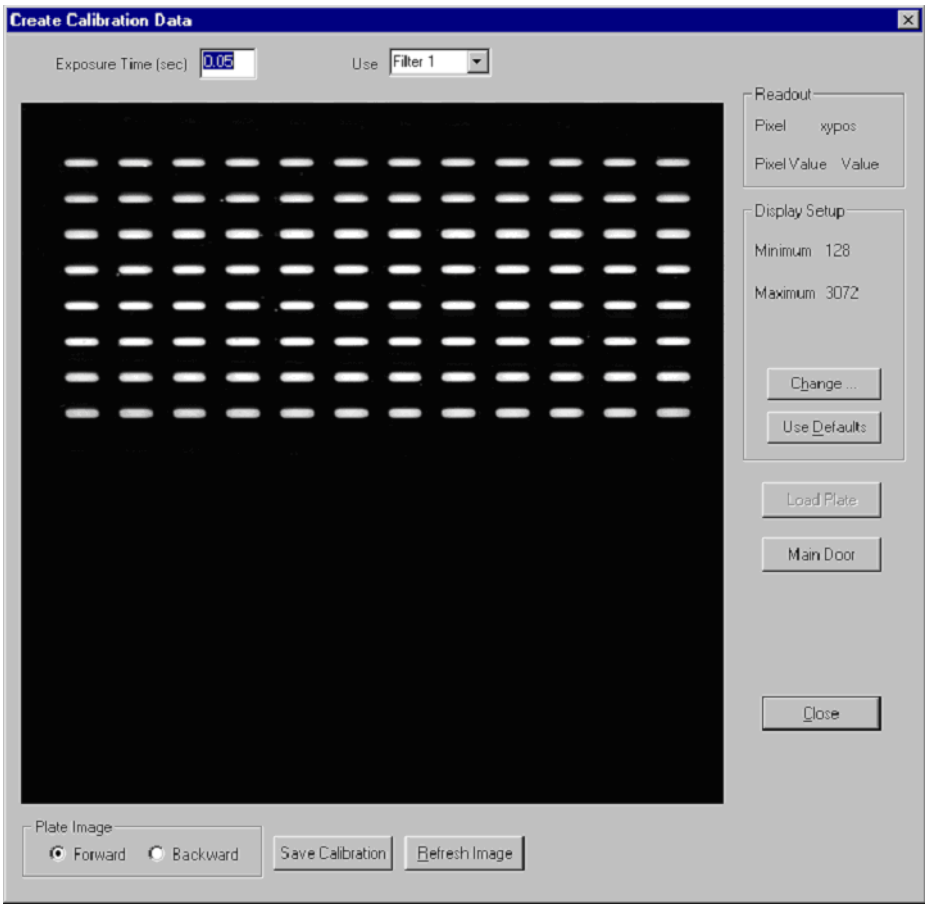
This appears when you choose **Calibration** from the **Setup** menu.

The calibration process causes the software to examine the optical properties of a plate and correct for minor variations in the optics of the system. Once the calibration is performed, the information is stored in the software and then applied to every plate run in the system.

It is recommended to always use a calibration when running an assay because it will greatly enhance the precision and reproducibility of the results.

A new calibration should be performed when:

- Changing assay types *e.g.*, luminescence to fluorescence
- Adding a new plate format
- Having a problem with imaging output.
- Setting up the FLIPR system



Item	Description
Exposure Time	Enter the length of time (in sec) for the aperture of the camera to remain open while taking an image.
Use	Select the appropriate filter to use for the exposure from the drop-down list.

Item	Description
Close	Click **Close** to close the window. No data will be saved if you have not run a calibration plate.
Readout	This panel provides the pixel information about the image. **Pixel**—This is the total number of pixels in the entire image. **Pixel Value**—When holding the arrow cursor over the image, the intensity value of the pixel directly beneath the arrow cursor is displayed in this area.
Display Setup	This panel allows you to adjust the minimum and maximum range of the signal values. **Minimum**—minimum range of the signal. **Maximum**—maximum range of the signal. Clicking **Change** allows you to change the minimum and maximum range values. Anything below the minimum signal range will display as black and anything above the maximum range will display as white. Everything within the signal's operating range will display as gray. Clicking **Use Defaults** resets the minimum and maximum range values to the default values.
Main Door	This button acts like a toggle to open and close the main instrument door. This allows you to place and remove a plate from the stage.
Refresh	Click **Refresh** to take a new image.
Plate Image	This panel informs the software the orientation of the plate when creating a calibration. To create a proper calibration file, both orientations should be included. **Forward**—This refers to the orientation of the plate such that well A1 is in the upper-left corner as you are facing the instrument. **Backward**—This refers to the orientation of the plate that is turned 180° from the forward position.
Create Calibration Data	Click this button to create a calibration file from the currently displayed image.

Performing a Calibration

To perform a calibration:

Step	Action
1	Choose **Calibration** from the **Setup** menu.
2	Click **Main Door** to open the door.
3	Insert the calibration plate in the forward position.
4	Click **Main Door** to close the door.
5	Make sure that the **Forward** option is selected in the **Plate Image** panel.

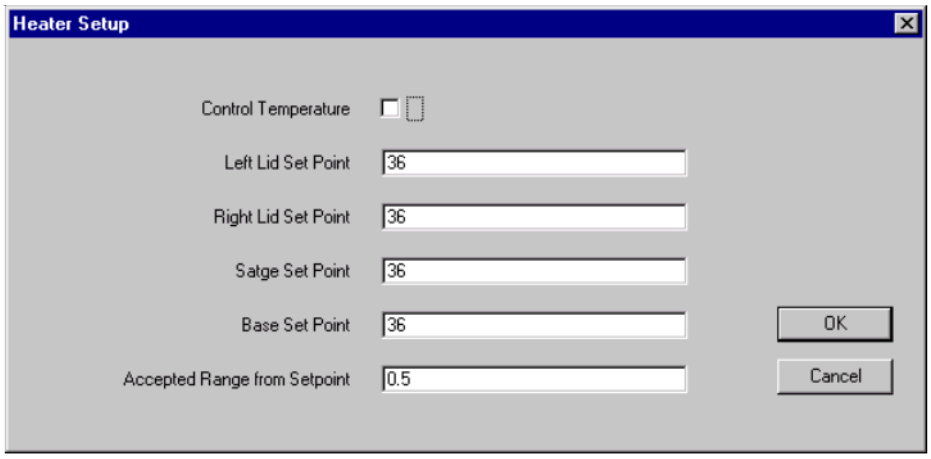
To perform a calibration: *(continued)*

Step	Action
6	Click **Refresh Image**. When the calibration is finished, a dialog box will appear asking you to reverse the plate.
7	Open the door and turn the calibration plate 180°.
8	Close the door and select the **Backward** option in the **Plate Image** panel.
9	Click **Refresh Image**.
10	Click **Save Calibration** to create the calibration file before closing the dialog box.

Heaters Dialog Box

This appears when you choose **Heaters** from the **Setup** menu.

This dialog box allows you to set up the temperature control of a heated stage. You must have the heated stage installed and the option selected in the **Hardware** dialog box to use this feature.

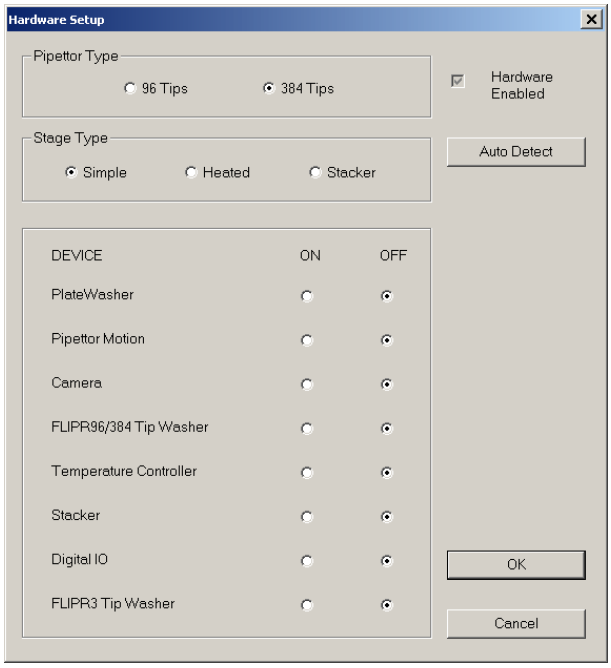


Option	Description
Control Temperature	Check this option if you want to control the temperature of the stage. If this box is not checked, the stage heater will not be turned on.
Left Lid Set Point	Enter the desired temperature (in °C, range X–X) for the lid in Position1.
Right Lid Set Point	Enter the desired temperature (in °C, range X–X) for the lid in Position 2.
Stage Set point	Enter the desired temperature (in °C, range X–X) for the stage.
Base Set Point	Enter the desired temperature (in °C, range X–X) for the stage base.
Accepted Range from Setpoint	Enter the number of degrees that you will accept as a variation from the set point.

Hardware Dialog Box

This appears when you choose **Hardware** from the **Setup** menu. The options in this dialog box allow you to:

- Manually turn hardware components on and off
- Check communication between the software and hardware
- Change the setting for the stage and pipet head

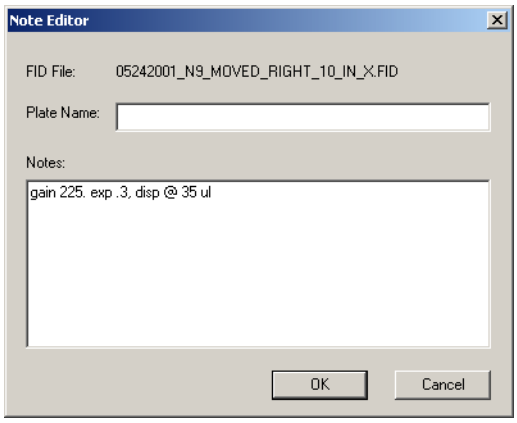


Item	Description
Pipettor Type	Specifies which pipettor head is installed. **IMPORTANT** Do not change the **Pipettor Type** setting. The pipettor head must be changed only by a Molecular Devices Service Representative. If you need to change the pipettor head, please call Molecular Devices to schedule a service call.
No Hardware	Check this option when you are running the FLIPR system software without a connection to the instrument (*e.g.*, on your desktop). This temporarily disables the hardware.The hardware is turned on the next time the hardware setup dialog is opened.
Stage Type	Specifies which **Stage Type** is being used. Click the option that corresponds to the appropriate stage. **Note** If you change the stage, then the pipettor settings must be rechecked.
Device	Displays each hardware component and specifies the current **ON/OFF** state. Allows you to change the current hardware state.
Auto Detect	Click this button to direct the software to check all devices and display their current **ON/OFF** state.

View Menu Dialog Boxes

Notes Editor Dialog Box

This appears when you choose **Notes Editor** from the **View** menu. This allows you to enter comments that are specific to the plate you are running.



View Experiment Dialog Box

This appears when you choose **Experimental Setup** from the **View** menu. The **View Experiment** dialog box is very similar visually to the **Experiment Setup** dialog box. In the **View Experiment** dialog box, the parameters cannot be changed. The purpose of this dialog box is to be able to review the experiment protocol used to produce the data. You may click the **Edit** button to load the protocol into the **Experimental Setup** dialog box to create a new protocol or to save a new control file.

Software Error Messages

Error Codes and Messages

FLIPR software error codes and explanations:

Error Code	Error Message/Explanation
401	The Data directory drive does not have enough free space. Please choose another drive.
402	The Data directory drive does not have enough free space. Please choose another drive.
403	The Export directory drive does not have enough free space. Please choose another drive.
404	The Export directory drive does not have enough free space. Please choose another drive.
801	Air supply OFF
901	Prime: Error priming tip washer from Source Bottle A. The bottle may be empty.
902	Prime: Error priming tip washer from Source Bottle B. The bottle may be empty.
903	Experiment halted due to error draining reservoir during wash.
904	Fill from Source Bottle casued overflow. Please check the system.
905	Experiment halted due to problem with tip wash bottles.
906	Fill: Error priming tip washer from Source Bottle A. The bottle may be empty.
907	Fill: Error priming tip washer from Source Bottle B. The bottle may be empty.
908	Experiment halted at user request due to full tip wash waste bottle.
909	Tip washer priming halted due to drain bottle full.
910	Tip washer priming halted due to bottle error.
911	Waste bottle (A or B) is full.
912	Tip washer overflow indicated.
913	Tip washer source bottle is empty or the waste bottle is full.
914	User requested continuation, but tip wash waste bottle is still full.
1002	Restore fluid halted at request of user - all recovery tasks aborted.
1003	Experiment halted during presoak at operator's request; fluid returned to plate.
1004	Instrument halted at request of user.
1005	Set Camera Gain halted at request of user.
1101	Invalid file name.
1301	Unable to backfill to Source Bottle A. Please check the system.
1302	Unable to backfill to Source Bottle B. Please check the system.
1401	The aspiration setup for this system requires a 384 head. The setup files indicate that a different head is installed.
1401	The dispense setup for this system requires a 384 head. The setup files indicate that a different head is installed.
1401	The dispense setup for this system requires a 96 head. The setup files indicate that a different head is installed.

FLIPR software error codes and explanations: *(continued)*

Error Code	Error Message/Explanation
1401	Pipettor and presoak task types do not match.
1401	The setup for this system requires a 384 head. The setup files indicate that a different head is installed.
1401	The setup for this system requires a 96 head. The setup files indicate that a different head is installed.
1402	Aspiration plate and head type do not match.
1402	Dispense target plate and head type do not match.
1402	Target plate to put tips in and head type do not match.
1403	Head and aspiration plate type are same format. Quadrant pipetting is not possible.
1404	Camera temperature can be cycled only for the FLIPR^3 system.
1404	Set Camera Gain can be performed only with the FLIPR^3 camera.
1501	The heated stage must be installed to perform this experiment.
1601	Filter door is open.
1701	Unable to move big cylinder to up position before dumping tips.
1701	Unable to return big cylinder to up position after dumping tips.
1701	Unable to move big cylinder to fully up position before loading tips.
1701	Unable to move big cylinder from fully up position during load tips.
1701	Unable to return big cylinder to fully up position after loading tips.
1701	Unable to move big cylinder to up position before unloading tips.
1701	Unable to move big cylinder from fully up position.
1701	Unable to return big cylinder to up position after unloading tips.
1702	Unable to move big cylinder to fully down position to dump tips.
1702	Unable to move big cylinder to fully down position during load tips.
1702	Unable to move big cylinder to fully down position during unload tips.
1801	Experiment halted due to error in moving to plate to aspirate.
1801	Instrument halted while moving to tip unload.
1801	Experiment halted due to error in move to plate during clear pipet head.
1801	Dispense halted due to error in move to plate.
1801	Error in setup causing invalid move to plate command.
1801	Error detected in move to plate step.
1801	Error in moving to plate to put tips in well.
1801	Experiment halted due to error putting tips in well.
1801	Experiment halted due to error in moving to tip washer.
1802	Dispense halted due to error in mixing.
1802	Experiment halted due to error in cycling head during wash tips (Source A).
1802	Experiment halted due to error in cycling head during wash tips (Source B).
1802	Experiment halted due to error in cycle head during wash.
1901	Unable to open right clamshell on heated stage, experiment halted.

FLIPR software error codes and explanations: *(continued)*

Error Code	Error Message/Explanation
1902	Unable to open left clamshell on heated stage, experiment halted.
2001	Halting experiment because the instrument timed out when checking temperature zones.
2001	Halting experiment at user request while waiting for heated stage to reach temperature.
2101	Unable to close main door, experiment halted. Please check the door.
2101	Instrument has halted because it is unable to close the main door to dump tips.
2101	Instrument halted due to error in closing main door prior to homing pipettor.
2101	Instrument halted due to error closing main door prior to loading tips.
2101	Instrument has halted because it is unable to close the main door to unload tips.
2102	Unable to close door after stopping experiment - recovery steps halted.
2201	Instrument has halted because it is unable to open the main door after dumping tips.
2201	Unable to open main door to load plates from stacker.
2201	Instrument halted due to error in opening door before manually loading stacker.
2201	Unable to open main door, experiment halted. Please check the door.
2201	Unable to open main door to unload plates to stacker.
2202	Unable to open door after stopping experiment.
2301	Invalid directory.
1102	User defined string for file name contains illegal characters for a file name.
1103	User defined string for file name is too long.
1103	No name for the data file generated is indicated.
2401	The plate assigned to the read position has no mask. Please specify a different plate.
2401	The plate assigned to the read position has no mask. Please specify a different plate.
2402	Error in input to Assign Filter for All Imaging.
2403	Cannot read barcode of read plate because it is not loaded by the stacker.
2403	Cannot read barcode of source plate because it is not loaded by the stacker.
2404	You have requested including a user-defined string in the file name but have not provided one.
2404	A user-defined string has been provided but is not included in the file name.
2404	You have requested use of a barcode in a name but not requested its entry.
2404	Cannot include user-defined string None in the file name.
2405	Default plate name provided to the notes editor is too long.

FLIPR software error codes and explanations: *(continued)*

Error Code	Error Message/Explanation
2406	An aspiration height, volume, or hold volume is invalid.
2406	Hold volume and aspiration volume cannot sum to greater than 28. Please adjust values (384-well head).
2406	Hold volume and aspiration volume cannot sum to greater than 255. Please adjust values (96-well head).
2406	Hold volume and aspiration volume cannot sum to greater than 30. Please adjust values (384-well head).
2406	Hold volume and aspiration volume cannot sum to greater than 255. Please adjust values (96-well head).
2406	Please adjust stroke volumes in tip wash steps.
2407	Trying to aspirate from a position with no plate.
2407	Trying to dispense to a position with no plate.
2407	Trying to presoak in a position with no plate.
2407	Trying to put tips in well in a position with no plate.
2408	The minimum aspiration height for plate X is Y. Please adjust aspiration height for this plate.
2408	The maximum aspiration height for plate X is Y. Please adjust aspiration height for this plate.
2408	The minimum dispense height for plate X is Y. Please adjust aspiration height for this plate.
2408	For plate X, the minimum presoak height is Y and maximum is Z. Please adjust the presoak height for this plate.
2408	Please adjust height to put tips in this plate.
2409	The maximum speed for aspiration in this system is X. Please enter a lower value.
2409	The maximum dispense speed in this system is X. Please enter a lower value.
2409	The maximum presoak dispense speed in this system is X. Please enter a lower value.
2409	Please enter a lower pipettor speed for tip washing.
2410	The maximum expel volume in this system is X. Please enter a lower value.
2410	Mixing after dispense requires that both Mix Cycles and Volume be greater than zero.
2410	The maximum expel volume in this system is X. Please enter a lower value.
2410	The maximum presoak expel volume in this system is X. Please enter a lower value.
2410	Please enter a lower value.
2412	Default note provided to the notes editor is too long.
2413	In Remote Control Mode, only a single bar code may be entered.
2414	The Start image for Export Statistic #1 must be less than (or equal to) the Last Image.
2414	Export Statistic #2 - Requested Start Image is #. It must be less than (or equal to) total # of images (#).

FLIPR software error codes and explanations: *(continued)*

Error Code	Error Message/Explanation
2414	Export Statistic #2 - Requested Last Image is #. It must be less than (or equal to) total # of images (#).
2414	The Start Image for Export Statistic #3 must be less than (or equal to) the Last Image.
2414	Export Statistic #3 - Requested Start Image is #. It must be less than (or equal to) total # of images (#).
2414	Export Statistic #3 - Requested Last Image is #. It must be less than (or equal to) total # of images (#).
2415	Export of data in quadrant form is not possible in 96-well experiments.
2416	Cannot prompt user for export file name if remote control is ON.
2701	Experiment halted due to error in loading tips
2702	Exiting experiment due to error in Aspiration.
2704	Instrument halted upon error during unloading tips.
2705	Halting experiment as a result of errors in machine status check.
2706/2707	Experiment halted due to error in dispensing.
2708	Instrument halted due to error in aspiration during presoak.
2709/2710	Instrument halted due to error in dispense during presoak.
2712	Unable to wash tips after stopping experiment. Recovery steps halted.
2713	Error in unloading tips after stopping experiment. Recovery steps halted.
2714	Experiment halted due to error in clearing stacker.
2901	Not available for remote control.
2902	Cannot prompt for file name in data export with remote control on.
3501	Priming: Communication error with tip washer.
3502	Filling: Communication error with tip washer.
3503	Backfilling: Communication error with tip washer.
3504	Draining: Communication error with tip washer.
3601	Unable to move plate slider into position to return plates.
3601	Unable to move plate slider into position to receive plates.
3601	Unable to move plate slider into position to return compound plate.
3601	Unable to move plate slider into position to return read plate.
3602	Unable to move read plate into position in stacker stage.
3701	Unable to return compound plate from stacker plate carrier to plate cartridge.
3701	Unable to return compound plate to plate cartridge.
3702	Unable to return cell plate from stacker plate carrier to plate cartridge.
3702	Unable to return read plate to plate cartridge.
3703	Plate already present on stacker plate carrier. Clear the stacker before loading read plate.
3703	Plate already present on stacker plate carrier. Clear the stacker before loading compound plate.

FLIPR software error codes and explanations: *(continued)*

Error Code	Error Message/Explanation
3704	Unable to load read plate - stacker cartridge appears to be empty.
3704	Unable to load compound plate - stacker cartridge appears to be empty.
3801	Attempting to aspirate when the tips are already filled with fluid will cause an error. Empty or unload tips.
3801	Tips are full. Empty or unload the tips.
3901	The default directory does not exist. Please enter a save directory that does exist.
3901	The directory entered as the save directory does not exist. Please re-enter.
3902	The Data directory is read-only. Please choose another directory.
3902	The default export directory does not exist. Please enter a save directory that does exist.
3903	The directory entered as the export directory does not exist. Please re-enter.
3904	The Export directory is read-only. Please choose another directory.
4001	Unable to move midpoint cylinder into blocking position before dumping tips.
4001	Unable to move midpoint cylinder into blocking position during load tips.
4001	Unable to move midpoint cylinder into blocking position before unloading tips.
4002	Unable to move midpoint cylinder from blocking position during load tips.
4002	Unable to move midpoint cylinder from blocking position before unloading tips.
4101	Unable to save file. Experiments with be halted.
4201	Unable to close left clamshell on heated stage, experiment halted.
4202	Unable to close right clamshell on heated stage, experiment halted.
4301	Digital IO is unavailable.

5 Running an Experiment

Introduction

In This Chapter

This chapter provides all the information and procedures required to perform an experiment on the FLIPR® system.

Overview

About Running an Experiment

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

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

These tasks are explained in detail in the remainder of this chapter.

Example Protocols

Following the general procedures for running, protocols are outlined for three types of assays:

- Calcium flux
- Membrane potential
- Aequorin (FLIPR^3 system only)

Assay Optimization

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

Preparing the Cells

Location of Cells in the Plate

The main optical feature of the FLIPR System is its bottom illumination and detection system which provides an excellent signal-to-background ratio. The depth of field of the camera is approximately 200 µm, which means that the fluorescence is measured at a depth of 200 µm from the bottom of the wells. For this reason, the cells must be located at the bottom of the wells during the assay. Adherent cells are typically grown overnight in the cell plate, whereas non-adherent cells are seeded on the day of the experiment and then spun down by centrifugation.

Cell Densities

Cell densities used in fluorescence assays vary because each cell type has different requirements. Cell densities range from 5,000 to 100,000 cells per well for 384-well plates and 20,000 to 400,000 cells per well for 96-well plates.

It is necessary to optimize the cell seeding density so that a uniform, 80%–90% confluent monolayer is formed on the day of the assay. Over-confluent cell monolayers 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.

Cell Seeding

Cells can be seeded in plates using a multi-channel pipettor or a liquid-dispensing system, such as the Labsystems Multidrop 384 or the Matrix PlateMate. We recommend seeding a 384-well cell plate using an automatic instrument rather than seeding manually. The thin needle used in automatic liquid-dispensing instruments prevents the formation of air bubbles at the bottom of the wells—a problem commonly encountered when the cells are seeded with a manual pipettor. 96-well plates can be seeded by either manual or automatic methods. Please refer to your specific instrument's *User Manual* for instructions on how to dispense the cell suspension into the wells.

Cells are seeded in clear, flat-bottom, black-wall 384- or 96-well tissue culture plates. The flat bottom ensures that the cellular fluorescence is localized to a single horizontal plane. Adherent cells are seeded the day before the experiment, while non-adherent cells are plated on the day of the experiment. All steps are carried out in the same black-wall 96- or 384-well plate.

Note Flat-bottom, clear-wall tissue culture plates can also be used with the FLIPR Calcium assay Kit and Membrane Potential Assay Kit. Either black-wall or white-wall plates can be used when running aequorin assays.

Powering-Up the System

About Powering-Up FLIPR

To ensure that the system is functioning properly, make sure that you power-up the system well before you begin the experiment. This will give the laser, camera, and heater (if using) ample time to reach their optimal operating conditions.

Powering-Up FLIPR

Follow the start-up procedure in Chapter 3, *"System Start-up and Shut-down Procedures."* Make sure the appropriate stage and pipettor head are in place. If necessary, change the stage and enter the modifications in the **Hardware Setup** dialog box (see Chapter 1, *"System Overview,"* for details on the **Hardware Setup** dialog box).

Note Please contact Molecular Devices if you need to change the pipettor head. Do not attempt to do so yourself.

If temperature regulation is desired, check to see that the heated stage is installed. Then turn it on by activating the **Heaters** option in the **Setup** menu. Allow the same amount of time as the laser warm-up for complete temperature equilibration, and check the instrument status bar to ensure that the desired temperature is stable.

Checking the System

About Checking the System

Once a day, before running your first assay plate, run a Yellow Plate Test to ensure that the system is performing according to specifications.

Note Make sure that the instrument is warmed up before running a Yellow Plate Test or an assay.

CAUTION Store the yellow plate in a safe place away from bright light on an even surface. Avoid scratching the bottom of the plate, since that can affect the coefficient of variation.

Note You do not have to run a **Yellow Plate Test** more than once a day.

Running the Yellow Plate Test

To run a Yellow Plate Test:

Step	Action
1	Set the following parameters: For 384-well plate— a. Install the ledge support in the cell plate position (no mask). b. Adjust the laser power to 600 mW using the **Up** and **Down** arrows on the remote control pad. c. Make sure that the laser is set in **Light** mode. d. Set the camera's f/stop (aperture) to f/2. Check by looking at the camera lens through the right side door. e. Set the **Exposure Length** to 0.05 sec in the **Experiment Setup** dialog box. f. Set the camera gain to 10. For a 96-well plate: a. Place a slit-shape mask in the cell plate position. b. Adjust the laser power to 600 mW using the **Up** and **Down** arrows on the remote control pad. c. Make sure that the laser is set in **Light** mode. d. Set the camera's f/stop (aperture) to f/2. Check by looking at the camera lens through the right side door. e. Set the **Exposure Length** to 0.05 sec in the **Experiment Setup** dialog box. f. Set the camera gain to 10.
2	Place the yellow plate on the stage in Position 1.
3	Take a picture by selecting **Signal Test** from the **Run** menu. Acceptable yellow plate results are a standard deviation less than 3.5% and fluorescence between 10,000–20,000 counts. If the test results are outside of the range of acceptable values, perform the laser alignment procedure

To run a Yellow Plate Test: *(continued)*

Step	Action
4	Print the results and keep them in a "Maintenance" folder by the instrument to track the coefficient of variation of the yellow plate over time. Indicate on the results sheet the laser power wattage and amperage values, wavelength used, etc. **Note** The **Signal Test** and **Plate Viewer** pictures are not saved within a data file. In order to save the information, most users simply print the files. Alternatively, you can run an experiment with the yellow plate used as a "read plate" with no fluid addition scheduled. The yellow plate fluorescence count information will be stored in the data file. **Note** You do not have to run a Yellow Plate Test more than once a day.

Dye-Loading the Cells

About Dye-Loading

In order to be seen by the camera, the cells are incubated in the presence of a fluorescent dye. The dye can be calcium sensitive for intracellular calcium flux assays, or environmentally sensitive for membrane potential assays. Depending on the application of evaluation, the dye-loading step typically lasts no more than 1 hr (at either room temperature or 37° C).

Loading Duration and Temperature

The optimal loading time will depend on the cell type and whether the anion exchange protein inhibitor is used. Since both the fluorescent dyes and the anion exchange protein inhibitor are toxic to cells, it is best not to exceed the optimal loading time. If anion exchange inhibition does not enhance dye-loading conditions, it should not be used. A 60-min loading time at 37° C is usually effective for most cell lines and is the recommended starting point for 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.

Preparing the Compound Plate

When to Prepare the Compound Plate

Depending on its complexity, preparing a compound plate can take a variable amount of time. For this reason, it is best to plan your experiment carefully to ensure that the compound plate will be ready for use as soon as the cells are ready to be assayed.

Recommended Plates for Compounds

Polypropylene plates are recommended for inhibiting sticking of the compounds to the plastic surface. Using V-bottom 96-well plates to hold the compounds will minimize the dead volumes in the compound plate. Dead volume in 96-well compound plates is approximately 50 µL for flat-bottom plates and 10 µL for V-bottom plates. Dead volume in a 384-well flat-bottomed plate is approximately 25 µL.

Concentration of Compounds in the Plate

The compounds should be prepared at 3X, 4X or 5X the final concentration depending on the addition volume and the volume of buffer in the cell plate. These volumes may change, depending on the following considerations: the compound mixing efficiency, the adherence of the cells, and the kinetics of the cellular response.

Addition and Mixing of Compounds to the Cell Plate

Intracellular calcium and membrane potential measurements need to be performed quickly to capture rapid cellular kinetics. Therefore, it is necessary to define proper addition parameters (*i.e.*, compound addition speed, pipettor height, and volume) such that spontaneous mixing is achieved in the wells. The best mixture is achieved with high speeds, low pipettor heights, and high volumes. When determining these parameters by assay optimization, avoid the disruption or dislodging of weakly adherent or non-adherent cell monolayers. Adding compounds too rapidly can dislodge some types of cells from the plate. Larger 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 compound 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.

Note Typically the pipettor mixing feature is not used in the calcium or membrane potential assays. Due to the rapid signal updates, mixing during the rise of the signal could cause artifacts.

If the assay is run at a controlled temperature, the cell and compound plates should be brought to temperature outside of the instrument. It takes approximately 45–60 min in a dry incubator (15 min in a heating block) to warm the plates to equilibrium at 37° C.

Setting-Up an Assay Protocol

Using a Control File

The easiest way to use the FLIPR System control software is to make different types of control files (*.fcf files), and save them for future use. Some user groups prefer having a template file for each cell line, each project or each user.

If you already have control files, open the relevant *.fcf file by choosing **Open Control File** from the **File** menu and selecting the appropriate file from the dialog box. Alternatively, the bottom portion of the **File** menu lists the last four *.fcf Control files that have been used. This allows you to simply choose the file from the menu.

Creating a Control File

About Creating a Control File

Creating a control file requires defining the parameters of the experiment (see *"Installing FLIPR v. 2.1.1 Software for Desktop Computers*" on page 4-2 for details about the available options). These parameters include the following:

- Setting up the pre-assay conditions *i.e.*, plate positions, camera settings, filter, system checks, and file storage
- Creating the assay steps *i.e.*, the sequence of additions and imaging
- Choosing post-assay options *i.e.*, file naming conventions, file location

Defining the experiment parameters is done in the **Experiment Setup** dialog box located under the **Setup** menu.

All the tabs in this dialog box use the following conventions:

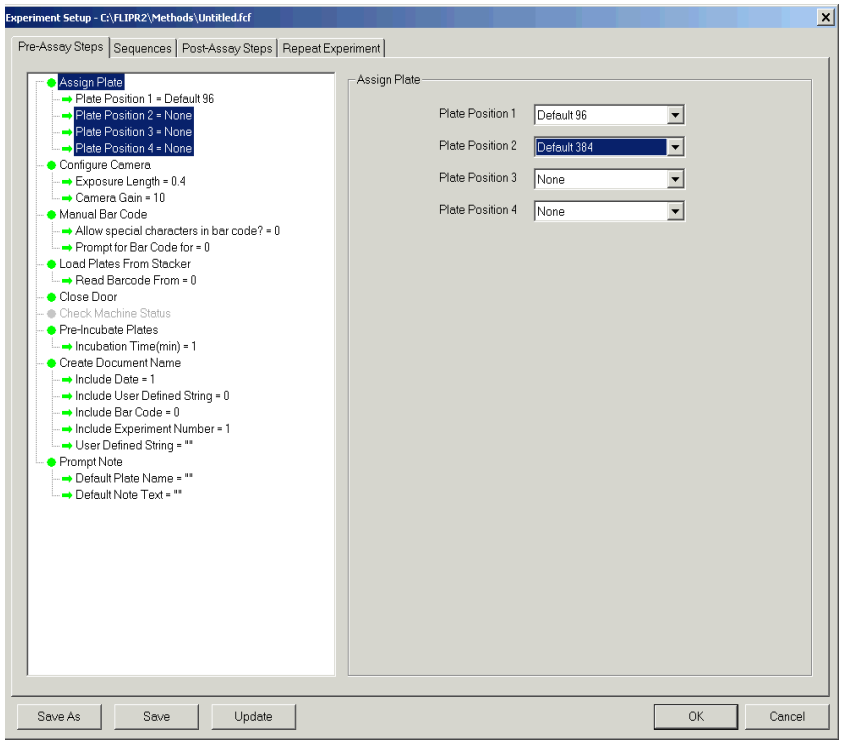
- All tasks marked with a green circle are active and will be performed in the order displayed on the list, with the exception of the **Sequence** tab (see note below).
- All tasks marked with a gray circle are inactive and will not be performed,
- A task may be activated or deactivated by double-clicking on it.

Note The tasks in the **Sequence** tab will be executed in the order listed up to the task of **Baseline Imaging**. After baseline imaging, all pipetting actions and imaging will occur in parallel.

Setting Up Pre-Assay Parameters

In the **Pre-Assay** tab of the **Experiment Setup** dialog box (located under the **Setup** menu), select the tasks on the left-side of the screen and enter the appropriate choices displayed on the right-side of the screen for the selected task.

For a detailed explanation of the experiment pre-assay tasks and their options see *"Pre-Assay Steps Tab"* on page 4-77.

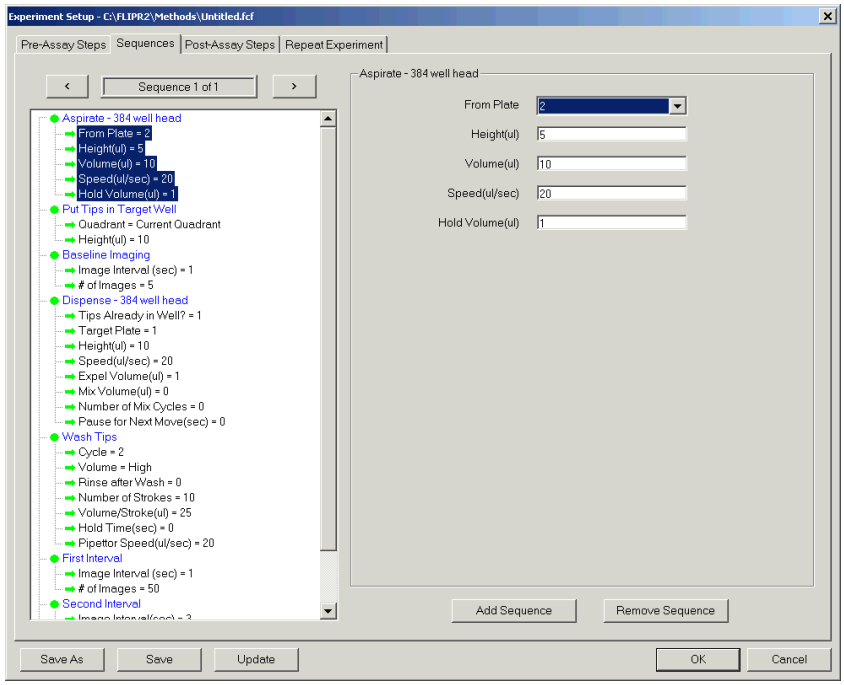


Creating the Assay Sequence

In the **Experiment Setup** dialog box, click on the **Sequence** tab.

Create the experiment sequence—select the task or action you want to perform and then enter the parameters of the selected action. For a detailed explanation of the **Sequence** tab tasks and their options, see *"Sequences Tab"* on page 4-85.

Note The tasks of the **Sequence** will be executed in the order listed up to the task of **Baseline Imaging**. After baseline imaging, all pipetting actions and imaging will occur in parallel.

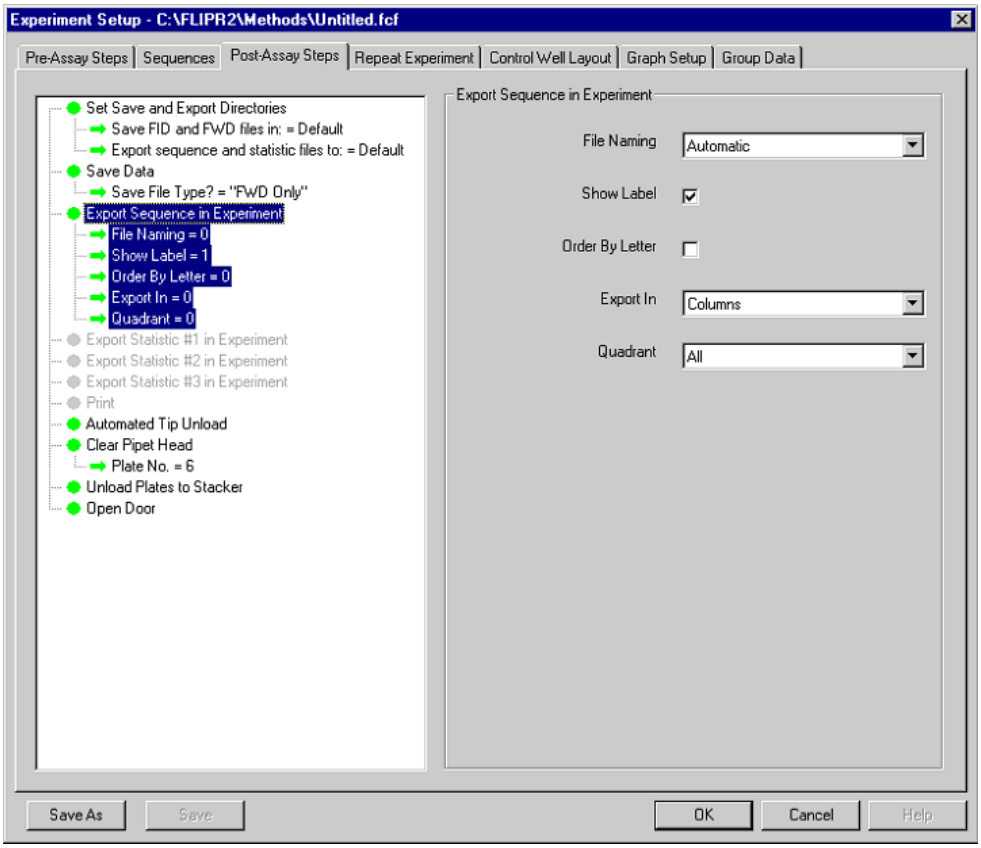


Choosing Post-Assay Options

In the **Experiment Setup** dialog box, click on the **Post-Assay Steps** tab.

Choose the location of where to save your experiment files and the naming convention to use for them.

For more information about the options of this tab, see *"Post Assay Dialog Box"* on page 4-96.



Repeat Experiment

Enter the appropriate options in this dialog box. See *"Repeat Experiment Tab"* on page 4-103 for more information on the options of this dialog box.

When Finished Defining Experiment Parameters

When you have finished defining the experiment parameters in the **Experiment Setup**, click **Save As** and save the file as an *.fcf file.

Optimizing the Signal and Fluid Dispensing

About Optimizing the Hardware Settings

Prior to running an assay, use the cell plate to check the following:

- Laser power
- Exposure time
- Camera f-stop (aperture)
- Camera gain
- Pipettor height
- Fluid dispensing speed
- Fluid volume

Optimizing Laser, Exposure, Gain and f-Stop

Note Although there are three parameters to adjust the basal signal to the desirable range, most users modulate two of the three parameters: the laser power and the exposure time. Most users keep the f-stop at f/2.

To optimize the fluorescence signal:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Place the cell and compound plates on the stage.</td></tr>
<tr><td>2</td><td>Place a tip rack in the tip pick-up position.</td></tr>
<tr><td>3</td><td>Start with the laser power, camera f-stop, gain and exposure time settings shown below. These settings are the most frequently used to measure basal fluorescence signal.
<table>
<tr><th>Settings</th><th>Membrane Potential Assay</th><th>Calcium Assay</th></tr>
<tr><td>Laser power (W)</td><td>0.600–1.20</td><td>0.600–1.20</td></tr>
<tr><td>Camera f-stop</td><td>f/4.0</td><td>f/2.0</td></tr>
<tr><td>Exposure time (sec)</td><td>0.4</td><td>0.4</td></tr>
<tr><td>Camera gain</td><td>100</td><td>100</td></tr>
<tr><td>Filter</td><td>540—590 nm bandpass filter</td><td>510—570 nm filter</td></tr>
</table></td></tr>
<tr><td>4</td><td>Perform a signal test to evaluate the basal fluorescence signal. The desirable fluorescence signal for intracellular calcium assays is 8,000–12,000 fluorescence counts. When running a membrane potential assay, the basal fluorescence counts should be between 20,000–30,000 counts to allow for a decrease or increase in the fluorescent signal.

If the basal fluorescence signal is substantially out of these ranges, the laser power, exposure time, gain and camera f-stop can be adjusted.</td></tr>
<tr><td>5</td><td>Increase the laser power if the basal fluorescence signal is too low or decrease it if the basal fluorescence signal is too high,

During the assay, the laser power should range between 0.600 W and 2.00 W.</td></tr>
</table>

To optimize the fluorescence signal: *(continued)*

Step	Action
6	If the basal fluorescence signal is too high, increase the f-stop from 2.0 to 2.8 or more. If the basal signal is too low, the laser power may be increased or the camera gain set higher. An f-stop value of 1.4 is the largest aperture opening setting for the FLIPR system.
7	If the basal fluorescence signal is too high, the exposure time can be decreased to a minimum of 0.05 sec. If the basal signal is too low, the exposure time can be increased, but the sampling interval will have to be increased to a minimum of exposure time + 0.3 sec (it takes 0.3 sec to integrate data).
8	Increase the gain if the basal fluorescence signal is too low or decrease it if the basal fluorescence is too high. The gain can vary between 1 (extremely bright fluorescence) to 255 (luminescence).

Adjusting the Pipettor Height

After the pipettor picks up fluid from the compound plate, it draws an air gap into the bottom of each pipet tip to ensure that fluid doesn't leak out. The bubble will be the first thing out of the pipet tip when fluid is dispensed. To avoid blowing bubbles in the wells (which can cause random light reflections and spurious signals), it is best to start dispensing with the tips above the fluid level in the wells. It is also preferable to have the pipettor tips submerged after the addition has been completed to ensure complete sample dispensing. If the pipet tips are in the air at the end of the fluid delivery, a drop can form on the end of the tip due to surface tension resulting in reproducibility problems. Therefore, the pipettor height should be set somewhere above the starting fluid volume in the wells, but below the final volume after the addition. For example, in 96-well format, if the wells contain 100 µL and the sample volume to be added is 50 µL, the pipettor can dispense the compounds from a height of 120–140 µL.

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

Adjusting the Fluid Dispensing Speed

The fluid default dispensing speed is 50 µL/sec for 96-well plates and 20 µL/sec for 384-well plates. The table below provides the limits for the fluid dispensing speed for both plate formats.

Cell Conditions	96-Well Plate (µL/sec)	384-Well Plate (µL/sec)
Slow dispensing speed for weakly adherent cells or non-adherent cells	10–40	5–10
Fast dispensing speed for strongly adherent cells	50–80	15–20

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

Larger 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 compound 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.

Start the Assay Run

To start the assay:

Step	Action
1	Check the **FLIPR Status Bar** to verify that the experiment synopsis is accurate and that the appropriate *.fcf file is being used.
2	Click the **Run** button on the toolbar to start the program. The main door automatically closes before any part in the FLIPR tower moves. The door remains closed until the end of the program including the duration of the pipettor tip wash.

Intracellular Calcium Assay Protocol Using the Calcium Assay Kit

About This Protocol

This protocol provides the following information and procedures:

- Materials required to run the assay
- 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:

Item	Source
FLIPR system with the 510–570 nm FLIPR emission filter installed	Molecular Devices Corp
Calcium Assay Kit	Molecular Devices Corp (P/N R-8033)
Clear, flat-bottom, black-wall or clear-wall 384- or 96-well plates	See Appendix X
Clear, polypropylene compound plate	See Appendix X
FLIPR pipette tips, 96- or 384-well	See Appendix A
Cells in suspension	–
Test compounds	–
Growth medium	Major laboratory supplier (MLS)
Incubator (5% CO2, 37° C)	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

The contents of the FLIPR Calcium Assay Kit are optimized to give robust results when used on the FLIPR system according to the protocol provided in the kit. The dye is excited at the 488 nm wavelength using FLIPR's argon ion laser. The signal is detected using the standard 510–570 nm emission filter included with every FLIPR system. Additional emission filters are not required.

Note The composition of the kit is proprietary and the subject of pending patents.

About Exchange Protein Inhibitors

Some cell types use mechanisms such as the anion exchange protein to export anionic molecules from the cells, including anionic forms of the fluorescent dyes. This will result in poor dye loading. Therefore, it may be critical for the success of the FLIPR system intracellular calcium assay to inhibit the action of the anion exchange protein.

Probenecid is an anion exchange protein inhibitor. When added to the loading buffer, it may increase dye retention in the cells. CHO 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 the cells, and therefore the inclusion and duration of the dye loading step should be kept to a minimum.

Cell Preparation for the Calcium Flux Assay

Cell Densities

Non-adherent cells are plated on the day of the experiment. We also recommend that you then centrifuge the plates at 1000 rpm for up to 4 min (**with the brake off**). Adherent cells are plated the day prior to the experiment and incubated in a 5% CO_2, 37° C incubator overnight. To create a 80%–90% confluent cell monolayer, we recommend seeding densities as shown below.

Cell Type (10^4 cells/well)	96-well Plate (100 µL growth medium)	384-well Plate (25 µL growth medium)
Adherent cells	2–10	0.5–3
Non-adherent cells	10–50	2.5–10

Dye Loading Using the Calcium Assay Kit

Preparation of Loading Buffer

The following procedure is designed for ten 96- or 384-well plates using either adherent or non-adherent cells prepared as described above.

To prepare loading buffer:

Step	Action
1	Prepare 1X Reagent buffer by pipetting 10 mL of 10X Reagent Buffer (Compound B in the Calcium Assay Kit) and diluting it to 100 mL with distilled water. Adjust to pH 7.4 with NaOH. **Note** Occasionally, a white precipitate will form in the 10X Reagent Buffer bottle. This is normal and will not affect the assay.
2	Remove a vial of FLIPR Calcium Assay Reagent (Component A of the Calcium Assay Kit) and equilibrate to room temperature. Dissolve contents of vial completely by adding 10 mL of 1X Reagent Buffer. Mix by repeated pipetting until the contents are completely dissolved. Prepare the Loading Buffer by diluting the vial mixture in 90 mL of 1X Reagent Buffer. Multiple washes of the vial are necessary to completely transfer the contents. **Note** 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, except as suggested in Chapter 9, "*Troubleshooting.*".
3	If your cells require probenecid, then a stock solution should be prepared freshly and added to the Loading Buffer to provide a final in-well working concentration of 2.5 mM. To prepare 250 mM probenecid stock solution (100X stock, enough for 1 liter of buffer), dissolve 710 mg probenecid in 5 mL of 1.0 N NaOH, and then mix with 5 mL of 1X reagent buffer. **Note** Do not store frozen aliquots of Loading Buffer with probenecid, and always add fresh probenecid the day of the experiment.

Loading the Cells Using the Loading Buffer

To load the cells:

Step	Action
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 plates).
2	Incubate cell plates for 1 hour at 37° C. In some cases, incubation at room temperature may work as well or better. **Note** The FLIPR Calcium Assay Kit is optimized for addition of antagonists as well. For 96-well plates, add 50 µL to each well and for 384-well plates add 25 µL. Addition volumes of agonist do not change, but fold concentrations and pipettor height do change.

Running the Calcium Assay on the FLIPR System

Recommended Setup Parameters

After incubation, transfer the plates directly to the FLIPR system and begin the calcium assay. Recommended experimental setup parameters are provided below.

Parameters	96-Well Plate	384-Well Plate
Compound addition volume (µL)	50	25
Compound concentration (fold)	5	3
Dispense speed (µL/sec) Adherent cells	50–100	10–20
Dispense speed (µL/sec) Non-adherent cells	10–20	5–10

Troubleshooting the Calcium Assay Kit

About This Section

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

Fluorescence Drop Upon Compound Addition

A drop in fluorescence may be the result of dislodging cells from the wells during compound addition. Lowering the addition speed could solve the problem in this case. Cells could be dislodged for different reasons. If the cells are weakly adherent, plating cells on poly-D-lysine coated plates should help. If more than one layer of cells has formed in the well due to over-seeding the cells, in the future seed fewer cells in the wells to avoid this problem.

If a fluorescence drop is still present after lowering the addition speed, then it may be the result of a dilution effect. Using the dye at a higher concentration (as in the parameters in the table below) has been shown to work in this case.

Parameters	96-well Plate	384-well Plate
Dilution volume for Dye Loading Buffer (mL)	50	66
Addition volume for Dye Loading Buffer (µL)	50	25
Compound concentration (fold)	4	4
Addition volume for compound (µL)	50	17

Different Compound Concentration

The concentration and the volume of the Dye Loading Buffer may be adjusted to accommodate different compound concentrations (if compound concentration is limiting). In all cases the cell supernatant volume is 100 µL for 96-well plates and 25 µL for 384-well plates. Use the table below as a guideline.

Plate	Loading Buffer		Compound	
	Dilution Volume (mL)	Addition Volume (µL)	Concentration (Fold)	Addition Volume (µL)
96-well	50	50	4	50
	33	33	3	67
384-well	66	25	4	17
	60	25	3	25

Serum-Sensitive Cells or Targets

Some cells are serum-sensitive, and this results in oscillations of intracellular calcium that could interfere with results. Also, some target receptors or test compounds might interact with serum factors. In these cases, the growth medium should be removed (and the cells may also need to be washed) prior to addition of Loading Buffer. The volume of growth medium removed should be replaced with an equal volume of 1X Reagent Buffer before loading. Alternatively, the cells could be incubated overnight in the medium containing lower concentrations of FBS (*i.e.* 0.5%) and not be washed prior to the addition of Loading Buffer.

Effects of the Buffer Used in the Assay

Although Molecular Devices recommends using 1X Reagent Buffer for most cell lines, some cell lines may not work well with this buffer. In these cases, different buffers should be evaluated to optimize the assay for the best performance. If the test compounds are proteins or peptides, it may be necessary to add 0.1% BSA in the compound dilution buffer to prevent the compounds from sticking to the plate.

Effect of DMSO on Intracellular Calcium Assays

High concentrations of DMSO are toxic to many cell lines and affect compound mixing. Also, DMSO can induce physiological changes in cells (*e.g.* 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 we have tested, there was no effect on signal level up to 1% DMSO final concentration.

The 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, DMSO may cause a calcium flux. In this case, add DMSO to the Loading Buffer so that the final concentration of DMSO in the wells will not change after buffer addition.

No Response

If there is no cell response, check to ensure that the cells were properly dye-loaded. This can be achieved by stimulating the cells with a ligand for endogenous receptors (*e.g.*, ATP, dUTP) or by using the non-specific calcium ionophore ionomycin.

Additionally, check to ensure that the buffer and dye-loading buffer contain sufficient concentration of calcium.

Membrane Potential Assay Protocol Using the Membrane Potential Assay Kit

About This Protocol

This protocol provides the following information and procedures:

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

Required Materials

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

Item	Source
FLIPR system with the 540–590 nm BP FLIPR Emission FIlter installed	Molecular Devices Corp (P/N 0310-4027)
Membrane Potential Assay Kit	Molecular Devices Corp P/N R-8034
Clear, flat-bottom, black- or clear-wall 384- or 96-well plates	See Appendix X
Clear polypropylene compound plates	See Appendix X
FILPR pipette tips, 96- or 384-well	See Appendix X
Cells in suspension	—
Test compounds	—
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

The contents of the FLIPR Membrane Potential Assay Kit are optimized to give a robust result when used according to the protocol on the FLIPR system. The dye in the kit is a lipophilic, anionic, bis-oxonol dye that can partition across the cytoplasmic membrane of live cells, dependent on the membrane potential across the plasma membrane. Its fluorescence intensity increases when the dye is bound to cytosolic proteins. When the cells are depolarized, more dye enters the cells, and the increased intracellular concentration of dye binding to intracellular lipids and proteins causes an increase in fluorescence signal. When the cells are hyperpolarized, dye exits the cells, and the decreased intracellular concentration of dye binding to lipids and proteins results in a decrease of fluorescence signal. The dye is excited at the 488 nm wavelength of the argon ion laser.

Note The composition of the kit is proprietary and the subject of a patent application.

Cell Preparation for the Membrane Potential Assay

Cell Densities

Use cell suspensions at 100 µL/well for 96-well plates and 25 µL/well for 384-well plates. Non-adherent cells are plated on the day of the experiment. It is recommended that 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 the 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 (10^4 cells/well)	96-well Plate (in 100 µL growth medium)	384-well Plate (in 25 µL growth medium)
Adherent cells	2–10	0.5–3
Non-adherent cells	10–50	2.5–10

Dye Loading Using the Membrane Potential Assay Kit

Preparation of Loading Buffer

The following procedure is designed for ten 96- or 384-well plates using either adherent or non-adherent cells prepared as described above.

To prepare loading buffer:

Step	Action
1	Prepare 1X Reagent Buffer by diluting 10 mL of 10X Reagent Buffer (Component B in the Membrane Potential Assay Kit) 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.
2	Remove a vial of FLIPR Membrane Potential Assay Reagent (Component A of the Membrane Potential Assay Kit). Dissolve contents of vial completely by adding 10 mL of 1X Reagent Buffer. Mix by repeated pipetting until the contents are completely dissolved. Prepare the Loading Buffer by diluting the vial mixture into 90 mL of 1X Reagent Buffer. Multiple washes of the vial are necessary to completely transfer the contents. **Note** 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 Loading Buffer

To load the cells:

Step	Action
1	Remove the 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 plates).
2	Incubate the cell plates for 1 hour at 37° C. In some cases, incubation at room temperature may work as well or better.

Running the Membrane Potential Assay on the FLIPR System

Installing the Filter

A filter must be installed in the Filter #2 position of the FLIPR system prior to running the assay.

To install the filter:

Step	Action
1	Remove the filter holder located inside the filter door of the FLIPR by releasing the two thumbscrews holding the filter holder in place, and slide the holder out onto a clean or towel-lined bench top. Filter #2 location should be empty.
2	Remove one ring by unscrewing in a counter-clockwise direction.
3	Carefully place the 540–590 BP FLIPR Emission Filter (P/N 0310-4077) in the #2 location and screw the ring back in place with the notches facing outward.
4	Place the filter holder in its correct position in FLIPR.

Recommended Setup Parameters

After incubation with the Loading Buffer, transfer the plates directly to FLIPR and begin the assay. Recommended experimental setup parameters are provided in the table below.

Parameters	96-well Plate	384-well Plate
Compound Addition Volume (µL)	50	25
Compound Concentration (Fold)	5	3
Addition Speed (µL/sec) Adherent Cells	50–100	10–20
Addition Speed (µL/sec) Non-adherent Cells	10–20	5–10

Note The FLIPR Membrane Potential Assay Kit is optimized for addition of antagonists as well as agonists. For 96-well plates, add 50 µL to each well and for 384-well plates add 25 µL. Addition volumes of agonist do not change, but concentrations and pipettor height do change.

Troubleshooting the Membrane Potential Assay Kit

About This Section

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

Fluorescence Drop Upon Compound Addition

When incubating cells for long time periods (*e.g.* over one hour), a large drop (5–10K fluorescent units) may occur upon compound addition on the FLIPR system. This drop is most likely due to dislodging cells from the wells during compound addition. Optimize the assay by shortening incubation times, plating cells on poly-D-lysine coated plates, and/or slowing FLIPR addition speeds.

Increase in Fluorescence

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

If there is no change in fluorescence, the assay may need to be redesigned. Recommendations include: longer incubation times and assay times, and choosing a different buffer, such as Tyrode's or specific ion-free buffers. For example, if studying a calcium channel, dye load the cells (using a calcium-free buffer for cell-suspensions) 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 (*e.g.*, 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.

Aequorin Assay Protocol Using the FLIPR System

About This Protocol

This protocol provides the following information and procedures:

- Materials required for the assay
- Cell preparation guidelines
- Coelentrazine loading procedure
- Running the assay on FLIPR
- Troubleshooting tips for Aequorin assays

Required Materials

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

Item	Source
FLIPR system	Molecular Devices Corp.
Clear, flat-bottom, white wall 384- or 96-well plates	See Appendix A, "Consumables Used With the FLIPR System."
Clear polypropylene compound plates	See Appendix A, "Consumables Used With the FLIPR System."
FILPR pipette tips, 96- or 384-well	See Appendix A, "Consumables Used With the FLIPR System."
Test compounds	—
Cells in suspension	—
Growth medium	Major Laboratory Supplier (MLS)
Incubator	MLS
Centrifuge	MLS
Pipettor and sterile tips suitable for use with microplates	MLS
1 N NaOH	MLS

Cell Preparation for Aequorin Assays

Cell Densities

Use cell suspensions at 100 µL/well for 96-well plates and 25 µL/well for 384-well plates. Non-adherent cells are plated on the day of the experiment. Adherent cells are seeded the day prior to the 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 (10^4 cells/well)	96-well Plate (in 100 µL growth medium)	384-well Plate (in 25 µL growth medium)
Adherent cells	2–10	0.5–3
Non-adherent cells	10–50	2.5–10

Coelentrazine Loading the Cells for Aequorin Assays

Loading the Cells

To load the cells:

Step	Action
1	Make up a 5 µM solution of coelenterazine in1X HBSS + 20mm HEPES + 0.1% BSA.
2	Add 100 µL (96-well plates) or 25 mL (384-well plates) of 5 µM coelenterazine to achieve a 2.5 µM final concentration.
3	Incubate the cells for 2 hrs at room temperature. Cover the plates with aluminum foil to avoid degradation of coelentrazine.

Running the Aequorin Assay on the FLIPR System

Removing the Filter

The filter slider must have one empty position in order to run aequorin assays.

Note We recommend Position 2 be empty.

To remove the filter:

Step	Action
1	Remove the filter holder located inside the filter door of the FLIPR by releasing the two thumbscrews holding the filter holder in place, and slide the holder out onto a clean or towel-lined bench top.
2	Remove one ring by unscrewing in a counter-clockwise direction.
3	Carefully remove the filter and screw the ring back into place with the notches facing outward.
4	Place the filter holder in its correct position in FLIPR.

Recommended Setup Parameters

Pre-Assay Conditions

Item	Parameter	Value
Assign Plates	Plate Position 1	96- or 384-well cell plate
	Plate Position 2	96- or 384-well compound plate (4X)
	Plate Position 3	
	Plate Position 4	
Camera	f/stop	1.4
	Exposure	0.3 sec
	Gain	225
Filter	–	2

Assay Sequence

Item	Parameter	Value
Aspirate	From Plate	2
	Height (µL)	10
	Volume (µL)	17
	Speed (µL/sec)	20
	Hold Volume (µL)	1
Put Tips in Target Well?	Height	35
Baseline Imaging	Image Interval	0.5 sec
	# of Images	5–10
Dispense	Target Plate	1
	Height	35
	Speed	20 for 384-well and 100 for 96-well plates
	Expel Volume	1
	Mix Volume	
	Number of Mix cycles	
	Pause for Next Move	
Wash Tips?		
First Interval	Image interval	0.5 sec
	# of Images	30
Second Interval	Image Interval	2
	# of Images	10

Optimizing an Assay

About Assay Optimization

The majority of the FLIPR system assay optimization is related to cell treatment prior to and during the assay. Cell and assay conditions that should be checked are listed below.

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

Cell Seeding

Conditions to check:

- Seeding density
- Seeding surface coating (collagen, poly-lysine matrix)
- Type of black-wall plate
- Serum-starvation
- Volume of growth medium in each well
- Growth medium replacement during seeding period

Dye Loading

Conditions to check:

- Dye loading buffer (inclusion of FBS or BSA, type of buffer)
- Dye loading temperature and duration
- Type of fluorescent dye
- Presence of additives (*e.g.*, probenecid and pluronic acid in intracellular calcium assays)

Compound Plate

Conditions to check:

- Type of buffer used (percent BSA used, for proteins and for peptides)
- Types of compounds used
- Concentration of compounds in the plate in relation to their final concentration in the assay (2X to 10X)
- Volume of fluid in compound plate in relation to required transfer volume and plate dead volume

Cell Washing

Conditions to check:

- Gentleness of the wash
- Manual vs. automatic washing method
- Consistency of volume left after the wash (particularly for intracellular calcium assays)

Preparing the Instrument

Conditions to check:

- Hardware check — optical alignment of laser and camera, calibration of pipettor height
- Illumination state — intensity of basal fluorescence
- Pipettor parameters — volume of compound added, height and speed of fluid addition, mixing *vs.* not mixing after addition

Using The Plate Stacker

6

Introduction

In This Chapter

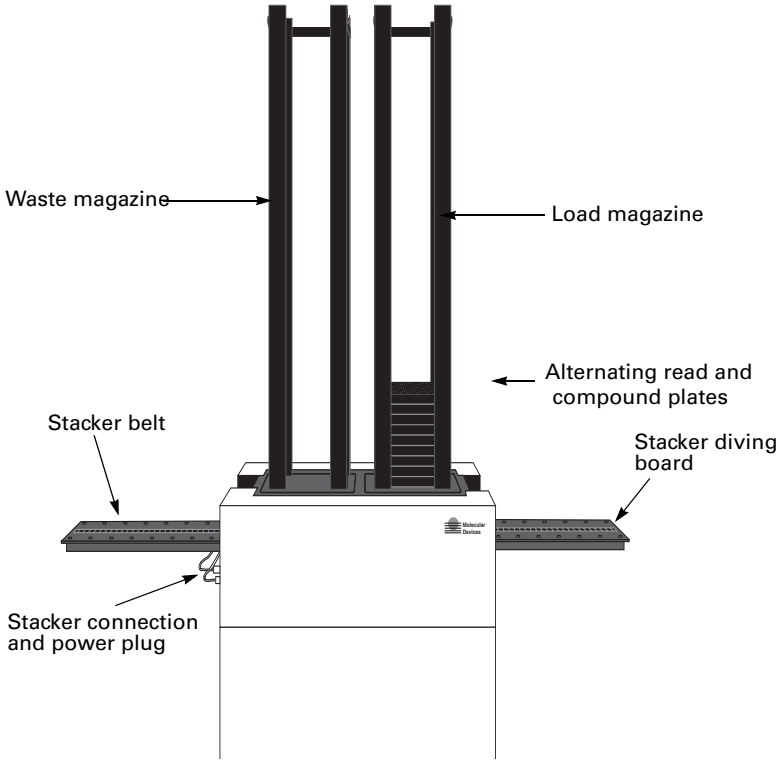
This chapter provides all the information and procedures required to operate the FLIPR system plate stacker.

Overview

About the Plate Stacker

The plate stacker (see below) is an accessory that can be integrated with the FLIPR system to increase assay throughput and allow hands-free operation. When running assays, multiple compound and cell plates can be stored on the stacker magazine and automatically shuttled into the FLIPR system. Alternating cell and compound plates are placed in the right side of the stacker magazine (called the “load” magazine), shuttled into the system and read. The plates are then carried across to the left side of the stacker magazine (called the “waste” magazine) and stored. Up to 52 cell and/or compound plates can be stored in one magazine.

This chapter provides a basic method for using the plate stacker with the FLIPR System.



Setting Up the Hardware

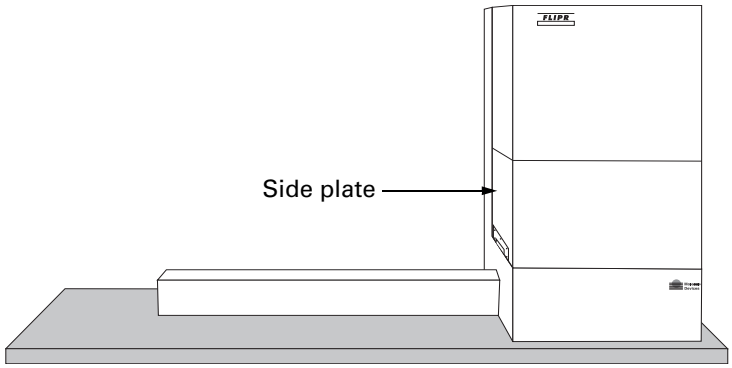
About Hardware Setup

This section explains how to set up the plate stacker and change from stage to stage.

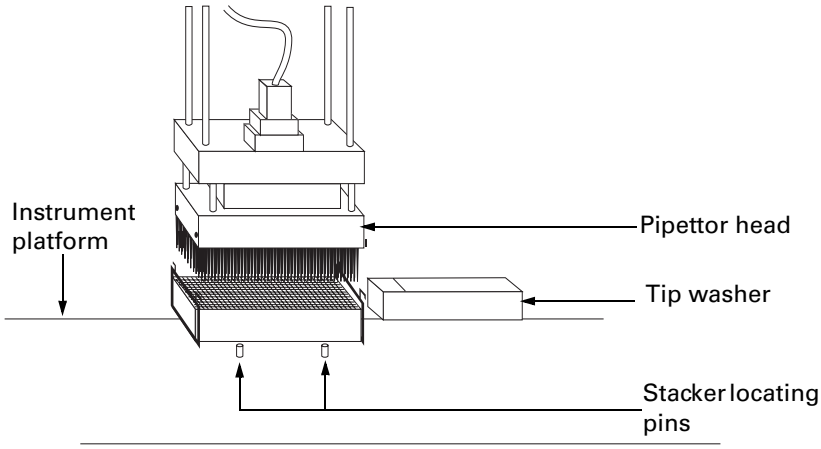
The initial installation of the plate stacker is performed by a trained Molecular Devices Service Representative. A platform is bolted in place above the FLIPR laser cover providing extra support for the stacker. During installation, the service representative will adjust the stacker position and air pressure for optimal operation.

Preparing the Stacker Unit

To prepare the stacker unit:

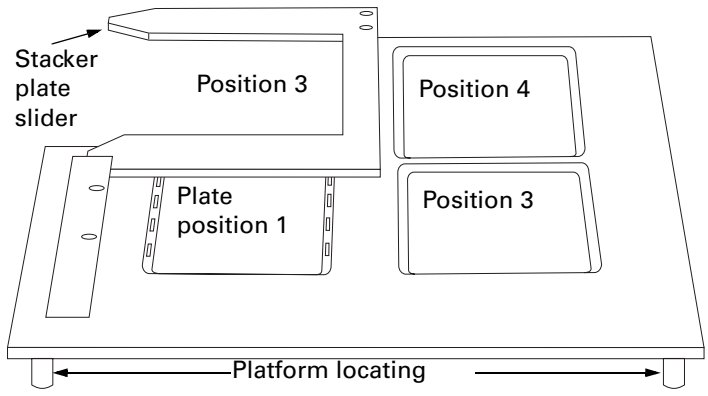
Body	Action
1	Turn on the FLIPR system, following the power-up sequence given in Chapter 3, *"System Start-up and Shut-down Procedures,"*. Make sure the main door is open.
2	Prior to any adjustment, remove the side plate facing the laser from the FLIPR instrument door. Removing this side plate allows the FLIPR door to close over the diving board during the assay while the plate stacker is in use. The side plate is held in place by five (5) Phillips-head screws on the left side plate of the instrument. Keep the side plate and the screws in a safe place. It will be necessary to replace them when the plate stacker is not in use. 

To prepare the stacker unit: *(continued)*

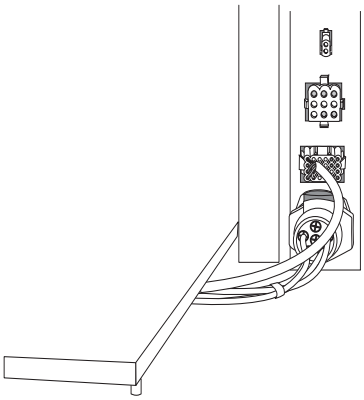
Body	Action
3	Position the plate stacker on the laser cover, which is located on the left side of the FLIPR instrument. The stacker belt should be parallel to the laser. Make sure the two reference pins located on the FLIPR platform fit into the two locating holes in the stacker belt. 
4	Connect the RS232 cable between the FLIPR instrument (the device #2 connection is located on the back of the instrument) and the plate stacker (located to the left of the plate stacker, near the power connection).
5	Connect the plate stacker to a power cord.
6	When the FLIPR control software is launched and the stacker is in power mode, the stacker initialization will take place first. This process takes approximately 35 sec and consists of testing belt movements and magazine pistons.

Installing the Stacker Stage

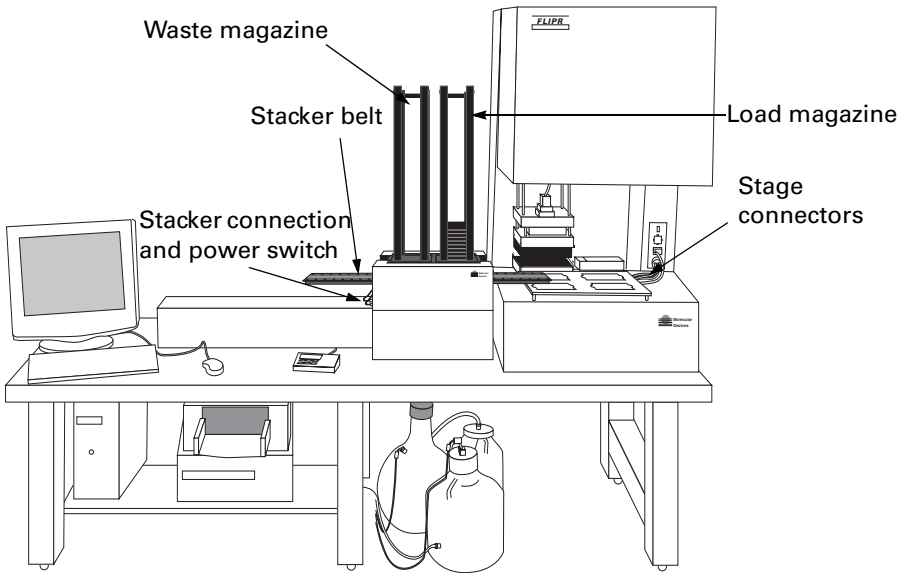
To install the stacker stage:

Step	Action
1	Place the stacker stage on the FLIPR system platform. Make sure that the stage locating pins fit in the platform holes. 

To install the stacker stage: *(continued)*

Step	Action
2	Connect the stacker stage to the FLIPR instrument by inserting the circular and square connectors into the matching plugs located above the stage. **Note** The simple and heated stages are not compatible with the plate stacker. 

FLIPR System with the Plate Stacker Installed



Removing the Stacker

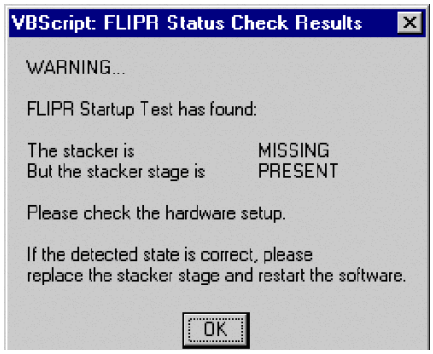
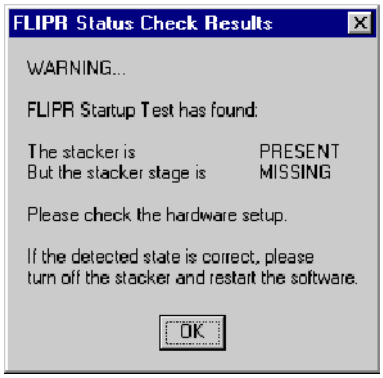
To remove the stacker:

Step	Action
1	Disconnect the stacker stage. The air connector may be unplugged by pressing down on the green release button.
2	Lift the stacker stage out of the instrument.
3	Lift the stacker up and carry it away from the instrument.
4	Screw the side plate back in place on the left side of the instrument panel.

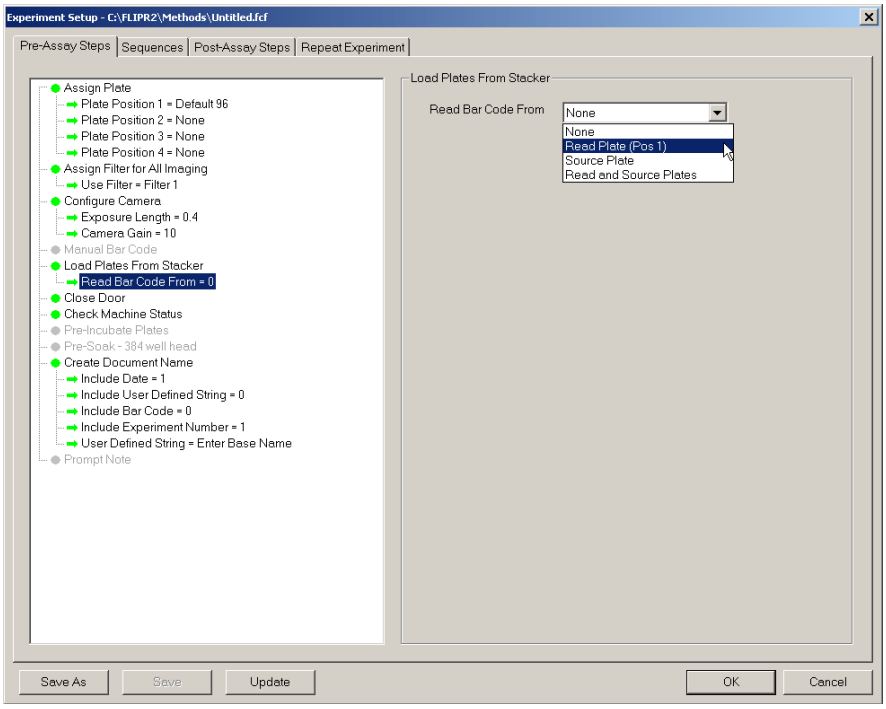
Setting Up the Software for the Plate Stacker Accessory

Setting Up the Plate Stacker in the Software

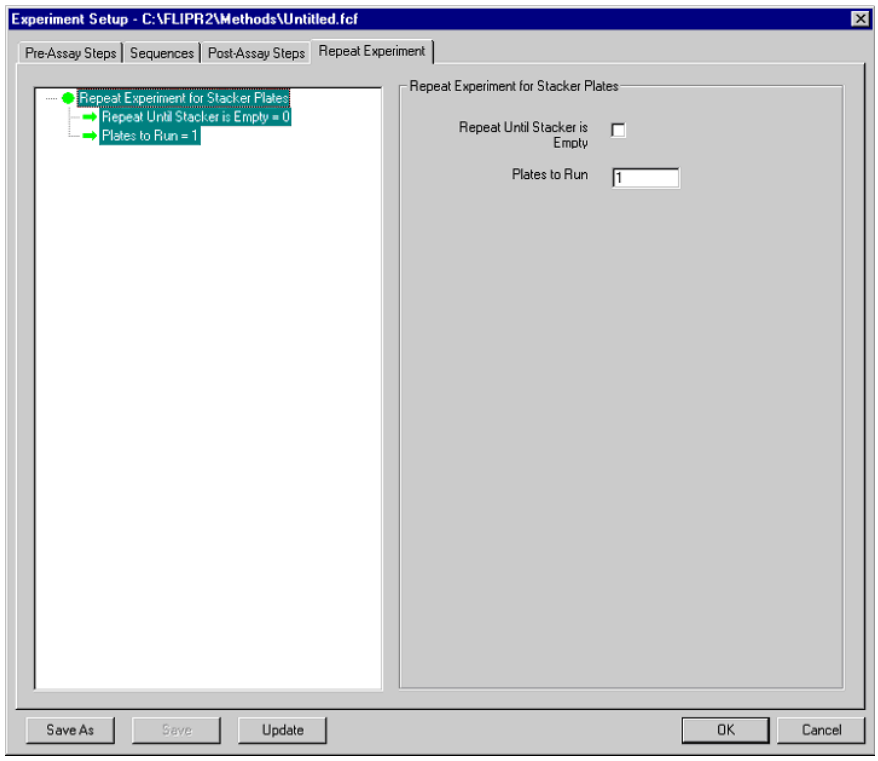
To set up the stacker in the software:

Step	Action
1	After installing the stacker, restart the FLIPR system software.
2	If the stacker is not installed properly, you will see one of the following error messages:   Check the connections, and restart the software.

To set up the stacker in the software: *(continued)*

Step	Action
3	After the stacker accessory is properly installed, the stacker options automatically appear in the **Experiment Setup** dialog box when creating or modifying a control file. The first option appears in the **Pre-Assay Steps** dialog of the **Experiment Setup**. If you are using bar codes, under the **Load Plates from Stacker** option, select the plate that contains the bar code from the **Read Bar Code From** drop-down menu. 

To set up the stacker in the software: *(continued)*

Step	Action
4	After identifying which plate has the bar code, click on the **Repeat Experiment** tab. If **Repeat Experiment for Stacker Plates** is dimmed, double-click on the option to enable it. Select one of the following options: ♦ Check **Repeat Until Stacker is Empty** to read all the plates or ♦ Enter the number of plates you want read in the **Plates to Run** field. **IMPORTANT** If you do not enable the **Repeat Experiment for Stacker Plates** option, only a single plate will be processed. 
5	Click **OK** to save the changes and close the dialog box.

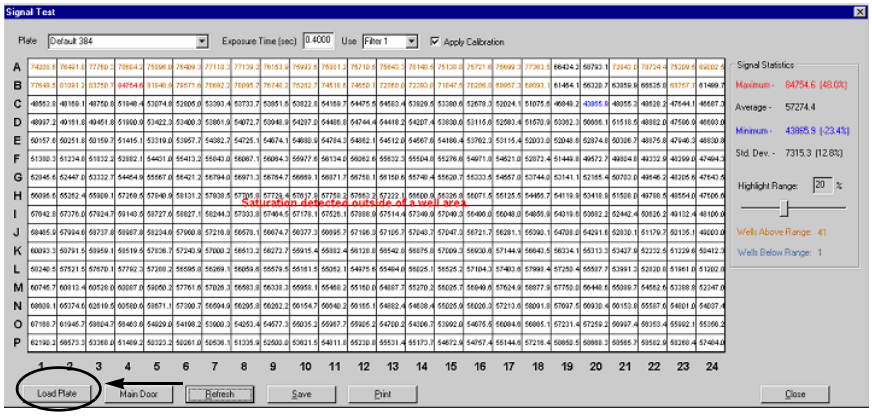
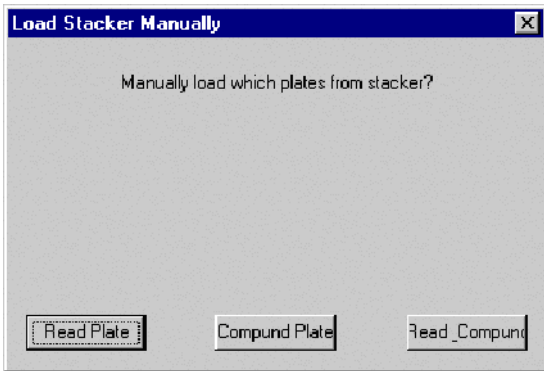
Running a Single Test Plate with the Stacker

The following procedure explains how to run a single plate using the plate stacker. This plate can be read using the **Signal Test**, **Plate Library**, or **Plate Viewer** options.

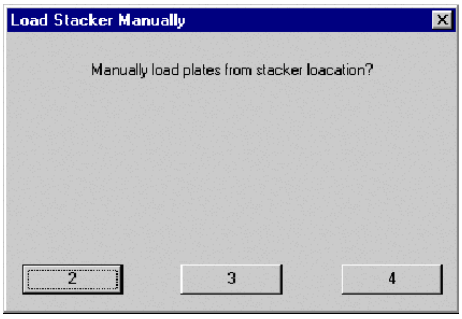
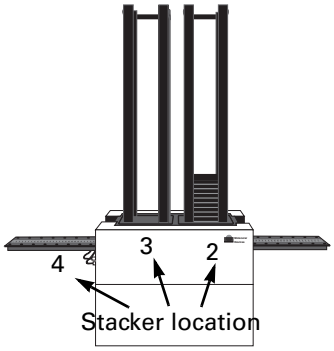
The procedure below uses the **Signal Test** as an example.

Note The yellow plate used on the stacker cannot have a lid. Lids are not compatible with the plate stacker. Do not use a FLIPR l yellow plate (which has an attached lid) on the stacker.

To run a single plate view using the stacker:

Step	Action
1	Select **Signal Test** or **Plate Viewer** from the **Run** menu. To check the alignment using the yellow plate, choose **Plate Library** from the **Setup** menu. Click **Load Plate**. 
2	In the **Load Stacker Manually** dialog box, click on **Read Plate**. 

To run a single plate view using the stacker: *(continued)*

Step	Action
3	In the **Load Stacker Manually** dialog box, click on the location of the plate you want to run. Use the schematic below to identify the locations.  
4	Load the plate in the appropriate position. Click **OK**. The stacker will load the plate. Click the **Refresh** button in the **Signal Test** dialog box to initiate a plate read. 

Running Experiments Using the Plate Stacker Accessory

Preparing Plates for the Assay

When the cell and compound plates are ready for the assay, alternate them in a stack on the bench starting with the first cell plate. Make sure no lids are used for any cell or compound plate. The last plate should be a compound plate. Place the stack in the load magazine.

Note Be careful not to contaminate the bottom of the cell plates when you slide the plates down in the magazine.

Cell Plate Signal Test

It is possible to run a signal test for the first cell plate. After the signal test function is activated, select **Signal Test** from the **Run** menu. See *"Running a Single Test Plate with the Stacker"* on page 6-9 for a procedure. After the signal test is complete, the assay can be started.

If all the plates in the magazine will be run in a row, a signal test cannot be performed before each assay. A signal test can be performed only for the first cell plate.

Running Experiments

The size of a plate batch depends on the cell type, the assay performed and the throughput needs. The current recommendation is for users to start with a batch of ten cell plates in a stack. Monitor them to see if the assay performance is affected by the lag time between the first and the last plate.

The plates in the load magazine are assayed and returned to the waste magazine where they are stacked in the reverse order.

Using the Plate Washer

7

Introduction

In This Chapter

This chapter provides all of the information and procedures required to operate, troubleshoot and maintain the FLIPR plate washer.

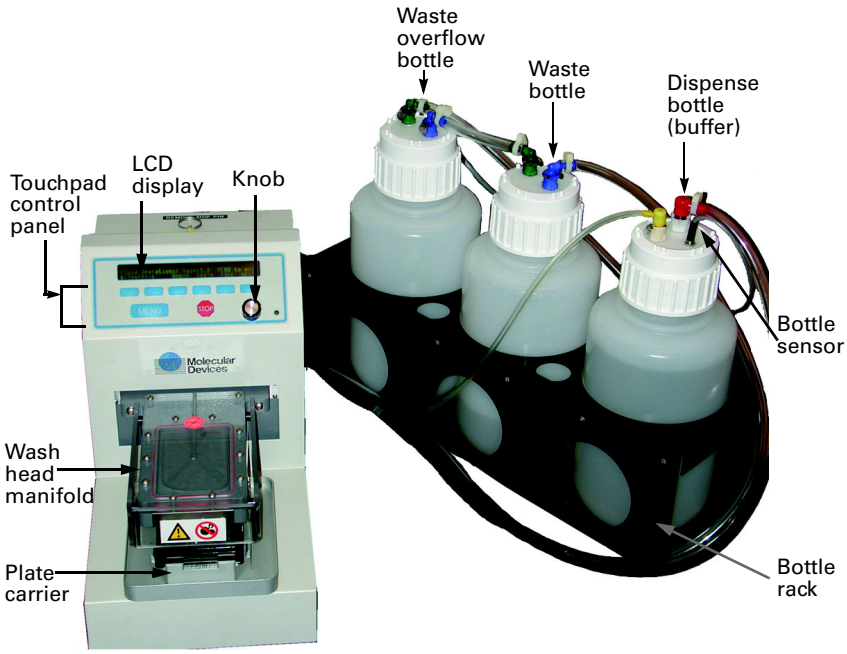
This chapter contains information on setting up and operating the FLIPR^{384} and FLIPR systems plate washer. Use it as a reference for daily operation and as a troubleshooting guide.

Overview of the Plate Washer

General Safety Precautions

Observe the following precautions when using the plate washer:

- Never put any objects under the wash probe manifold while the machine is running.
- Do not get the plate washer excessively wet. Unplug the machine and wipe up any spill immediately.
- Never open the panels while the washer is plugged in.



Features and Function of the Plate Washer

The plate washer can be used as a stand-alone unit or can be integrated to the FLIPR^{384} system to wash 96- and 384-well plates. The features and functions of the plate washer are listed in the table below.

Feature	Function
Quadrant positioner	Allows a single 96-probe manifold to wash both 96- and 384-well microplates.
On-board firmware	Stand-alone unit, no PC required. Touchpad programming to easily define, edit and store wash programs and plate types.
Store up to 10 plate types	Access to stored plate types eases transition between different 96- and 384-well microplates. Wash protocols are easily programmed.
Probe manifold XYZ positioning	User-definable dispense and aspirate heights to customize wash protocols to assay requirements.
Circular aspirate	To remove residual buffer adhering to the edges of 96-well plates.
Quadrant washing	Programmable quadrant washing for 384-well plates allows washing to be restricted to designated quadrants.
Wash programs	Store up to 40 customized wash programs.

Feature	Function
Soak mode	Primes the wash probe manifold at user-defined time intervals to prevent clogging of the dispense and aspirate pins.
Manual operation	Allows direct operation of the wash head without running a wash protocol.
Variable pressure and vacuum	Controls delivery speed by varying pressure; controls aspiration by varying the vacuum.
Instrument Setup	Touch commands to initialize the washer.

Setting Up the Plate Washer

Plate Washer Components

The following items are included in the plate washer shipment:

Item	Quantity
User Manual addendum	1
Base unit with wash head	1
Plate carrier	1
Bottle rack assembly	1
4 L buffer container with cap and fittings	1
4 L waste containers with cap and fittings	2
Bottle rack	1
Power cord	2
Vacuum pump	1
Base unit to electrical source	1
Base unit to vacuum pump	1
RS232 interface cable	1
Safety shield	1
Maintenance kit	1

After the instrument has been unpacked, confirm that all items listed on the packing slip are present.

When the plate washer is shipped, protective foam is placed around the unit to secure the XY axis motion control to ensure safe shipping. The plate washer is shipped in two boxes. One box contains the base unit, plate carrier and power cords. The second box contains the bottle rack assembly.

Unpacking the Base Unit

To unpack the plate washer base unit:

Step	Action
1	Remove the small box containing the plate carrier, power cords and cables.
2	Grasping the base unit from the bottom, lift the unit from the box and gently place the unit on a stable level surface.
3	Gently remove the foam from the base unit and lay the instrument on its side to expose the bottom of the instrument.
4	Remove the locking pin from the bottom of the unit. It is located in the rear of the bottom panel. **Note** The locking pin secures the XY axis motion control from being damaged during shipment. Keep the pin in a safe place in the event the instrument needs to be shipped (for instance, you can tape it to the side of the instrument).
5	Place the instrument upright and inspect it for damage such as cracks, dents or bent parts. **! WARNING ! If physical damage is found, contact Molecular Devices Service Department.**

To unpack the plate washer base unit: *(continued)*

Step	Action
6	The plate washer is now ready for setting up the bottle rack assembly. **Note** Save the packing material, which includes the box and foam. The original packing materials should be used if the instrument is returned or shipped to another location.

Unpacking the Bottle Rack Assembly

To unpack the bottle rack assembly:

Step	Action
1	Remove the entire bottle rack assembly from the box by lifting the black rack.
2	Remove the cardboard insert. It can easily be removed by disconnecting all tubing connections. To disconnect tubing, simply depress the metal collar inward to release each tubing connection. There are 3 bottles: ♦ 1 dispense buffer bottle, 4L ♦ 2 aspirate waste bottles, 4L **Note** Save the packing material, which includes the box and cardboard insert. The original packing materials should be used if the instrument is returned or shipped to another location.

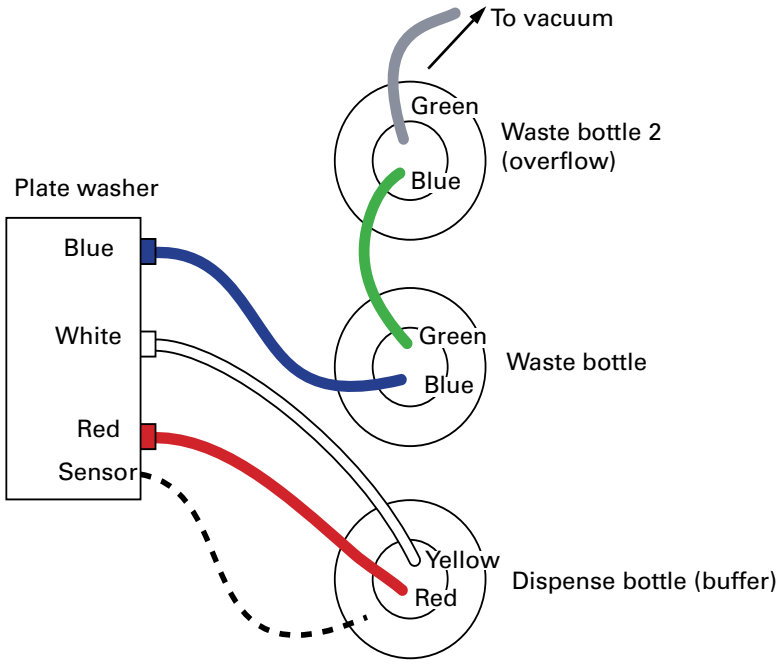
Installing the Plate Washer

Installing the Tubing

To install the tubing:

Step	Action
1	Reconnect the tubing connections to the appropriate bottles. The collars are color coded for easy identification and installation. Refer to the table and figure below for the color legend and for proper setup.

Tubing Connection	Color
Buffer	Red
Air	Yellow
Waste bottle inlet	Blue
Waste bottle outlet	Green



To install the tubing: *(continued)*

Step	Action
2	Connect the appropriate color-matched tubing to the rear of the plate washer and the vacuum. **Note** During calibration, the bottle assembly should be at the same level as the washer (*i.e.*, on the same bench). 
3	Ensure that the four set screws beneath the wash head manifold are securely fastened. If the screws are loose, it will affect how level the plate carrier is situated onto the base of the platform.
4	Place the plate carrier on top of the two placement pins beneath the wash head manifold. Ensure that the plate positioning spring is facing the rear end of the base unit and the "Front" marking is in front. **Note** The A1 position of the microplate must be located in the lower left front corner of the tray carrier.
5	Remove the locking pin located at the bottom of the washer. Make sure you keep it taped to the instrument, since you will need it to ship the washer.

Setting Up the Vacuum

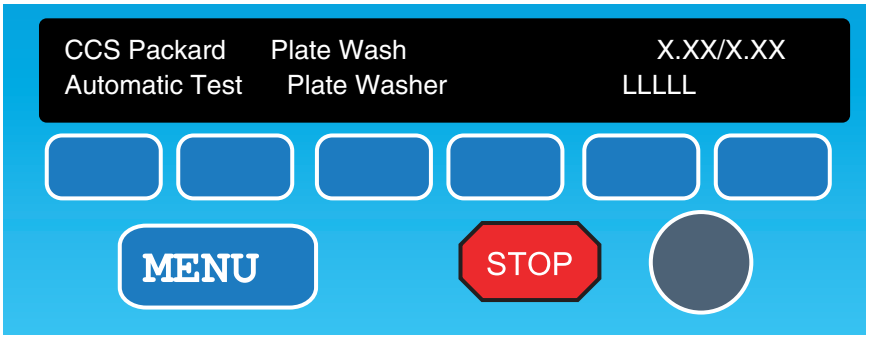
The plate washer requires a vacuum source for aspiration of fluid through the system.

To set up the vacuum:

Step	Action
1	Ensure that the green tubing is connected from the waste container to the labeled vacuum inlet of the vacuum pump.
2	Insert the supplied power cord into the vacuum electrical outlet in the rear panel of the plate washer.
3	Plug in the vacuum pump's electrical plug into the other end of the power cord for the vacuum. **Note** The plate washer supplies the electrical power to the vacuum pump. When the instrument is not in use, the vacuum pump automatically reverts to a "quiet mode" after a user-set time interval.

Powering Up

To power up the washer:

Step	Action
1	Before turning on the instrument, ensure that all the proper external connections have been made between the plate washer, bottle rack assembly and vacuum pump. Refer to *"Installing the Plate Washer"* on page 7-6 for a detailed description of the external connections. **CAUTION Make sure you have removed the locking pin located at the bottom of the washer and taped it to the instrument. It will be needed to ship the washer.**
2	Press the ON/OFF switch on the washer's rear panel. The screen will show the software version number in the upper right corner. In the lower right corner of the on-screen control panel, a short legend indicating the current operation will be displayed. 

Using the Control Panel

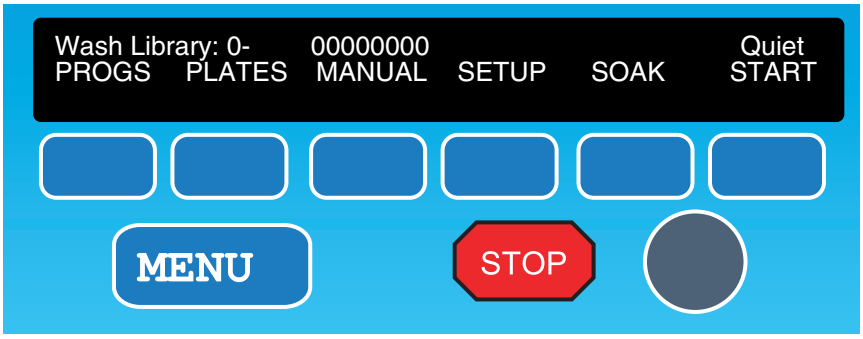
The Touchpad Control Panel

The plate washer contains on-board firmware that allows full control of the unit through the touchpad control panel. The touchpad control panel consists of a two-line fluorescent display with six unlabeled softkeys beneath it. During operation, labels on the lower line of the display define the functions of the softkeys. Only the labeled softkeys on any particular screen have any function. Pressing unlabeled softkeys will have no effect.

Panel Item	Function
MENU key	Beneath the touchpad control panel is the **MENU** key, which is generally used to return the plate washer to the previous screen or to exit a function without performing an action. At any time, continuously pressing the **MENU** key will eventually return the software back to the main screen.
STOP key	The red **STOP** key is used to halt any physical movements in progress. Generally, any action being performed by the plate washer will be immediately aborted by pressing this key. However, sometimes there may be a short delay before the current motion is terminated.
Knob	The "knob" is located at the right side of the control panel beneath the display screen. The knob simplifies programming by enabling users to scroll through a variety of options. Simply turn the knob in either direction for the following actions: ♦ Changing a numeric value ♦ Scrolling through selections in a list ♦ Entering an alphanumeric name Numerical values are frequently used in programming the plate washer. The values can sometimes span a large range. The knob utilizes dynamic acceleration to quickly make large changes when the knob is turned rapidly. When the knob is turned slowly, the effect of the knob becomes more precise, allowing the exact desired value to be reached in a short time.
Field Editing Indicator	A flashing block will appear in a field whose contents are being edited.
Power Source Indicator	A green pilot light at the extreme lower right corner of the control panel will light up whenever power is applied to the plate washer.

The Main Screen

All plate washer functions can be accessed from the Main screen. The top line of the Main screen shows the name and number of the current wash program. Turning the knob will change the program number, and the name of the associated program will immediately be displayed. No name will be displayed for wash programs that have not yet been defined.



The Main Screen Softkeys

The softkeys in the main screen provide access to the various support and programming functions of the plate washer. The main screen softkeys have the following functions:

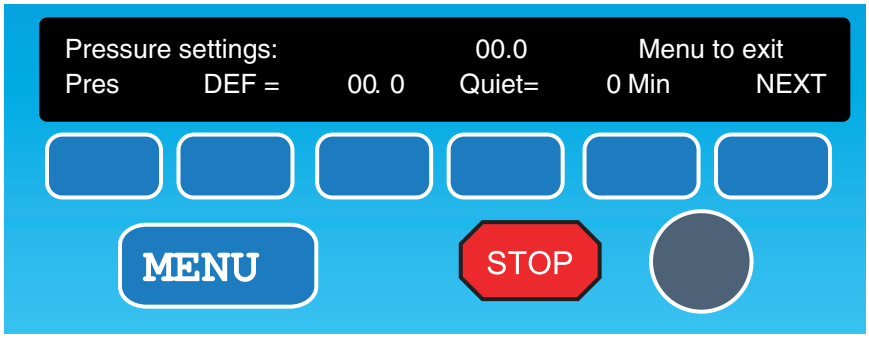
Softkey	Function
PROGS	Displays the **Program Library** screen from which wash programs can be created, edited, copied or deleted.
PLATES	Displays the **Plate Library** screen from which plate type definitions are created, edited, copied or deleted.
MANUAL	Displays the **Manual Plate** screen which allows for direct manual operation of the plate washer.
SETUP	Accesses functions for fluid and direct control, calibration, test, maintenance, initialization and communication configurations.
START	Allows you to start programs or commands.
SOAK	Maintenance option that leaves the manifold pins in wells containing deionized water and periodically cycles fluid through the manifold (in order to prevent the pins from clogging due to evaporation).

The Quiet Mode Screen

After the plate washer completes a wash program and returns to the main screen, the external vacuum pump and built-in pressure regulation system continue to run for a period of time. To reduce noise and wear on the system when not in use, a **Quiet Mode** can be set to start at a specific time after the wash program terminates. When the **Quiet Mode** starts, the external vacuum pump shuts off, and pressure in any of the fluid supply tanks is bled off to the

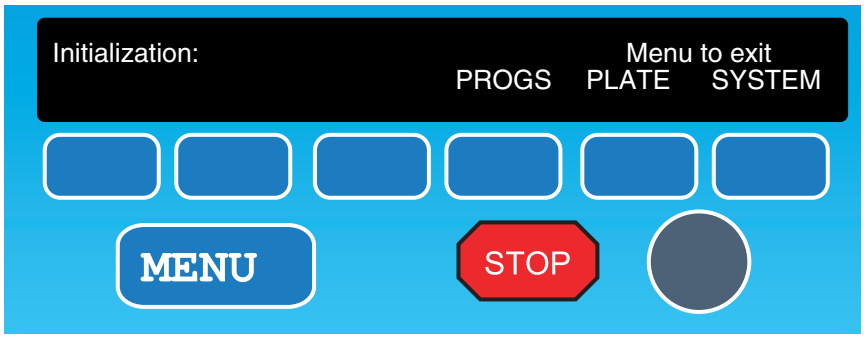
atmosphere. When in this mode, **Quiet** appears in the upper right corner of the display screen in the main screen.

To set the Quiet Mode time:

Step	Action
1	Press the **SETUP** softkey in the main screen.
2	Press the **FLUIDS** softkey.
3	Press the **NEXT** softkey.
4	Press the **QUIET** softkey and turn the knob to set the number of minutes idle time before the instrument goes into **Quiet** Mode.
5	Press **MENU** to exit. 

The Initialization Menu Screen

Pressing the main screen's **INIT** softkey displays the **Initialization** menu screen, in which plate libraries and system parameters can be cleared by pressing the associated softkey.



The Option Menu Screen

The **Option** menu screen contains several options that MUST be set prior to operating the plate washer.

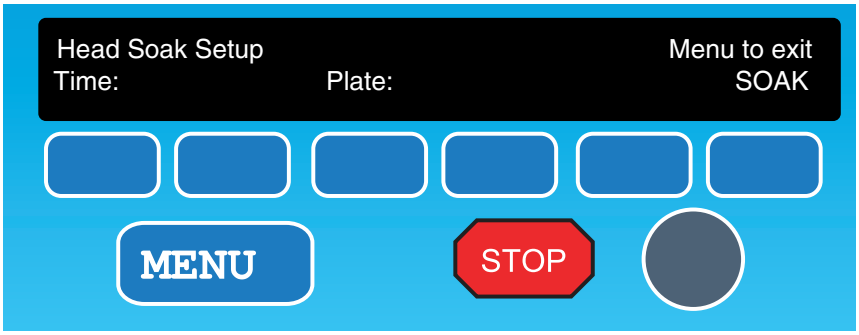
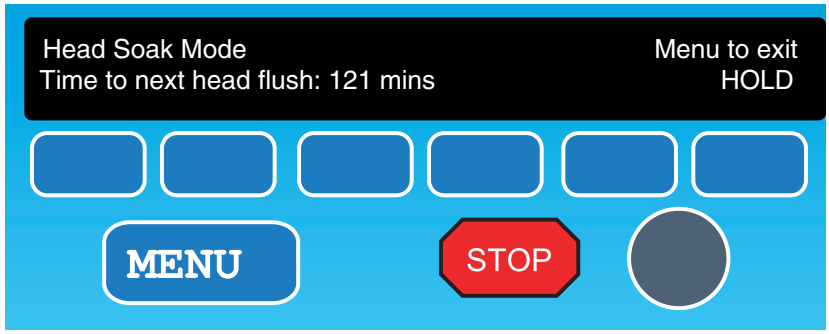
To set the proper options prior to running the plate washer:

Step	Action
1	**On low fluid detect:** press **FINISH**, **ABORT** or **IGNORE**
2	**Check for plate in place:** press **NO** or **YES**
3	**Minimum vacuum to allow run:** choose a value from **0.0** to **19.9**

The Head Soak Setup Screen

The **Soak** function primes the probes at user-defined time intervals to ensure that the dispense and aspirate probes are clean and unclogged while the instrument is not in use.

To set the soak function:

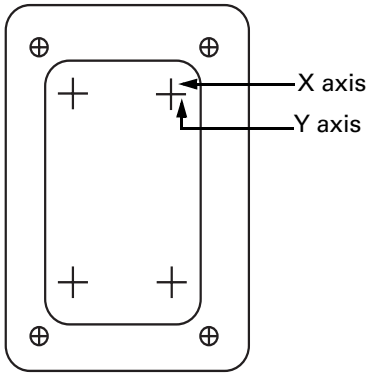
Step	Action
1	Press the **Soak** softkey in the main screen to open the **Head Soak Setup** screen. 
2	Press the **Plate** softkey. Use the knob to select a **Plate Type.** Place the microplate in the tray carrier.
3	Press the **Time** softkey. Use the knob to dial to the desired time interval (time is shown in minutes). The wash probe manifold will cycle through a dispense-aspirate-dispense procedure at the specified time interval. This will prevent crystallization or drying of compounds present in a wash solution, which would potentially clog the probes.
4	Press the **SOAK** softkey to begin the soak function. The display will change to the **Head Soak Mode** screen. 
5	Press the **MENU** softkey to terminate the soak function.

Setting Up the Plate Washer Following Installation

Homing the Plate Washer

The home position of the X, Y and Z axes MUST be established or verified prior to use or as needed for quality control. To set the home position relative to the wash head for the cell washer:

To home the plate washer:

Step	Action
1	Press the following softkeys, starting from the main screen: **SETUP → NEXT → OFFSET**.
2	Press the **Z** value softkey and use the knob to raise the wash probe manifold to 25 mm.
3	Place the plate carrier under the wash probe manifold on top of the two alignment pins.
4	Press the **Z** value softkey again and lower the wash probe manifold until it is just 0.1 mm above the surface of the plate carrier.
5	Press the softkey beneath the **X** value. Using the knob, position the wash head manifold until it touches the X axis cross hair. 
6	Press the softkey beneath the **Y** value. Using the knob, position the wash head manifold until it touches the Y axis cross hair. The probe should be touching the center of the cross hair.
7	Press the **HOME** softkey. The new **X**, **Y** and **Z** coordinates of the wash probe manifold will be stored as a **HOME** location. Once this **HOME** position is stored, the corresponding **X**, **Y** and **Z** axis values are set to zero. **Note** The straight (aspirate) pins are aligned to the carrier in the factory.

Leveling the Wash Head Manifold

The wash head manifold is leveled relative to the associated plate washer base unit prior to shipment. Prior to use (or as needed) the wash head manifold can be leveled using the procedure below.

To level the plate washer:

Step	Action
1	Place the plate carrier on top of the two alignment pins beneath the wash head manifold. Ensure that the plate positioning spring is facing the rear end of the base unit and the "Front" marking is in front.
2	Press the following softkeys: **SETUP→DIRECT→Z→ UP**.

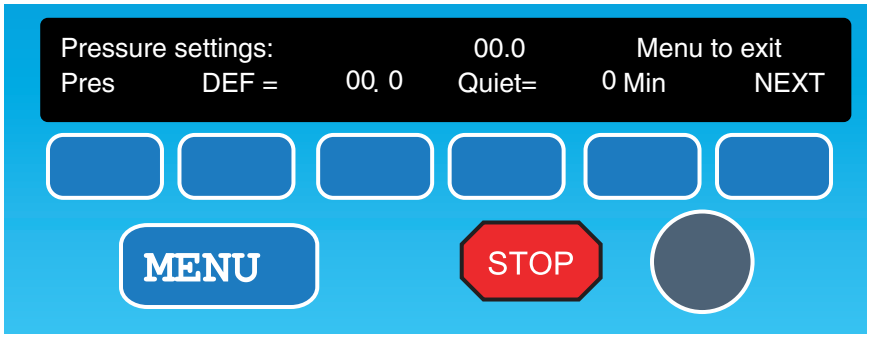
To level the plate washer: *(continued)*

Step	Action
3	Turn the knob until the probes are just 0.1 mm above the plate carrier. A 0.1 mm shim is provided to verify the leveling procedure. The shim should just fit beneath the wash probes.
4	Examine the four corners of the wash head manifold to check if they are all touching the plate carrier.
5	Use a flat head screwdriver on the white plastic screws to adjust the plate carrier height until all four corners are touching the pins. Counter-clockwise lowers and clockwise raises. After each adjustment, verify that the plate carrier does not wobble. It is critical that all four leveling screws be in contact with the base unit.

Defining the Wash Pressure

The pressure settings for the plate washer are fully programmable. In a wash program, the pressure is set to either **LOW**, **MED**, **HIGH** or **DEFAULT**. The functions for adjusting pressure settings are available under the **SETUP** softkey in the main screen.

To define the pressure settings:

Step	Action
1	Starting from the main screen, press the **SETUP**, **FLUIDS**, **NEXT** softkeys to display the **Pressure Settings** screen. The internal instrument pressure will be displayed on the top line. 
2	Use the knob to scroll to the desired pressure setting. There are four pressure settings: ♦ **DEF**: the default pressure setting ♦ **LO**: low pressure setting (recommended value 3 psi) ♦ **MED**: medium pressure setting (recommended value 5 psi) ♦ **HI**: high pressure setting (recommended value 7 psi)
3	Press the third softkey (under the number indicating the current value of the pressure setting selected in Step 2). Use the knob to adjust the pressure (pressure range 0.0 to 19.9 psi).

To define the pressure settings: *(continued)*

Step	Action
4	Repeat steps 2 and 3 until all of the pressure settings have been set. **CAUTION Although the pressure settings can be changed at any time, it is recommended to set them during the initial setup of the instrument. Changing pressure settings will affect the performance of wash programs that have already been optimized with previously established pressure settings. Confirm performance whenever the pressure settings are changed.**

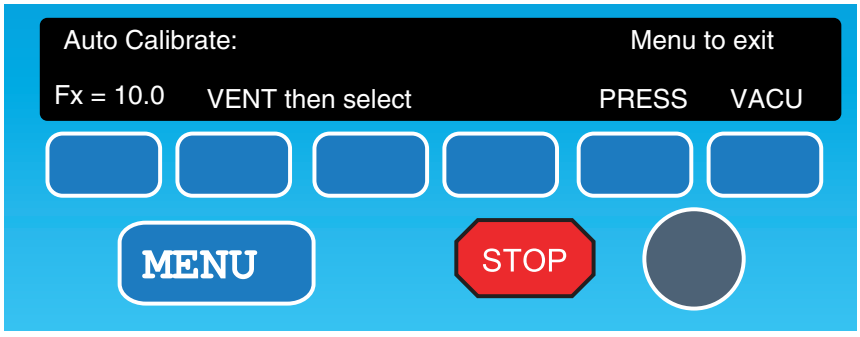
Calibration and Initialization

Prior to running the plate washer, it is essential that the instrument be calibrated for pressure and vacuum. "Fx" is a third setting that is calibrated in the factory and can be reset by the user. It controls the length of time a valve is open when fluid is dispensed. It is a function of tubing length and buffer viscosity. The following equipment is needed for the calibration process:

- Pressure gauge (supplied)
- Vacuum gauge
- Scale to measure weight in grams

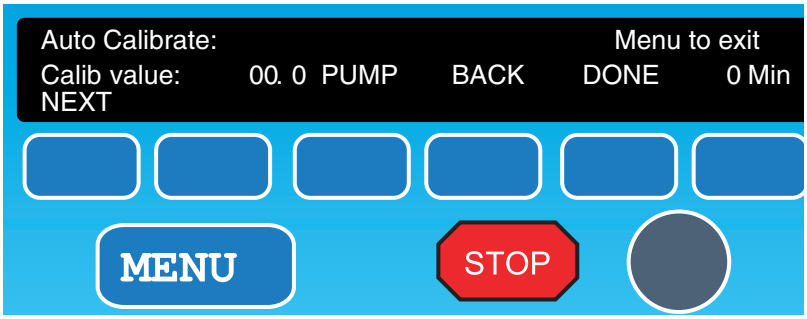
Preparing to Calibrate

To prepare for calibration:

Step	Action
1	Before calibrating, always make sure that the buffer bottles are at the same level as the washer (i.e., at the same bench height).
2	Starting at the main screen, press **SETUP** → **CALIB** to enter the **Calibration** screen. 
3	Press the **VENT** softkey. The bleed valve will release the air and vacuum out of the system.

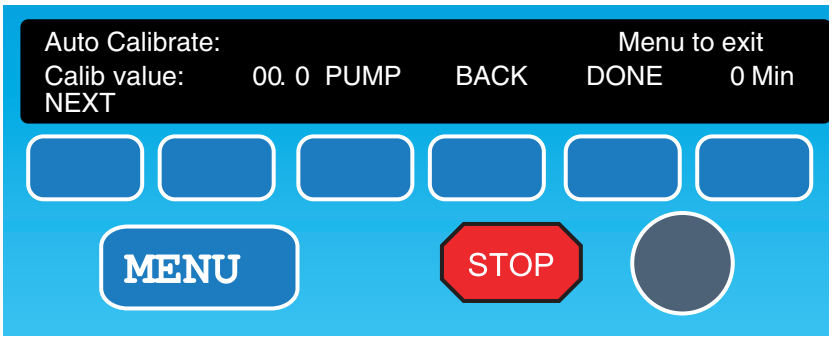
Calibrating the Vacuum

To calibrate the vacuum:

Step	Action
1	Ensure the plate washer has already been vented as described above.
2	Starting at the **Calibration** screen, press **VACU** to open the pressure/vacuum **Auto Calibrate** screen. 
3	Press the **PUMP** softkey. If using the recommended GAST pump, the gauge on the vacuum pump will rise to 15 Hg vac and remain there. Otherwise, obtain a vacuum gauge to insert between the vacuum inlet on the vacuum pump and the vacuum tubing to measure the vacuum present in the system. Wait until the vacuum pressure stabilizes before proceeding.
4	Turn the knob until the calibration value equals the value on the vacuum gauge.
5	Press the **DONE** softkey.
6	If the display reads "**Calibration Successful**", press the **BACK** softkey to return to the **Auto Calibrate** screen (if you want to calibrate the pressure), or press the **MENU** key to return to the main screen. If the calibration was not successful, press the **BACK** softkey and repeat the calibration process again.

Calibrating the Pressure

To calibrate the pressure:

Step	Action
1	Press the **PRES** softkey from the **Calibration** menu screen. The pressure/vacuum **Auto Calibrate** screen will appear. 
2	Remove the air tubing connection in the rear panel of the plate washer.

To calibrate the pressure: *(continued)*

Step	Action
3	Connect the air tubing to the pressure gauge and connect the other end of the pressure gauge into the air inlet in the rear panel of the plate washer.
4	Press the **PUMP** softkey. The pressure gauge will slowly rise. Let the gauge rise to a whole number (recommended value: 5.0 psi) and press the **PUMP** softkey again. The pump will turn off and the pressure gauge will indicate the value of the internal pressure.
5	Turn the knob until the calibration value (“**Calib value”**) on the screen equals the value on the pressure gauge.
6	Press the **DONE** softkey.
7	If the display reads “**Calibration Successful**”, press the **BACK** softkey to return to the **Auto Calibrate** screen (if you want to calibrate the vacuum), or press the **MENU** key to return to the main screen. If the calibration is not successful, press the **BACK** softkey and repeat the process.

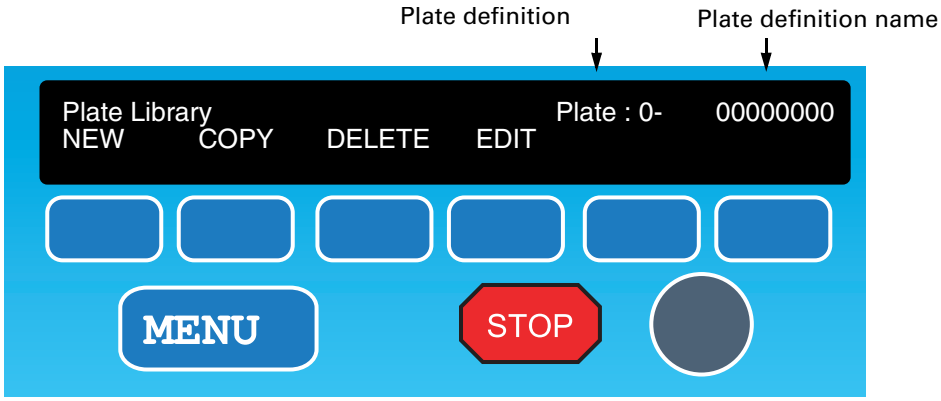
Calibrating Fx

Fx is a numerical factor used by the plate washer to compensate for the varying viscosities of fluids and the lengths of the tubes that carry the fluids from the reservoirs to the washer. Fx is calibrated in the factory and does not usually need to be re-adjusted by the user. The procedure for recalibrating Fx is detailed in Appendix D, *“Calibrating the Plate Washer.”*

Setting Up Plate Definitions

The Plate Library Screen

The plate washer can store up to ten different plate definitions numbered from 0 to 9. Users are able to access different plate definitions to quickly program a new wash protocol or switch to a different plate definition in an existing wash protocol. The **Plate Library** screen is shown below. Each plate definition is assigned a number and an alphanumeric name of up to 8 characters. Turning the knob, when in the **Plate Library** screen, scrolls through the plate definitions. The number and name of the chosen plate definition are displayed in the upper right corner of the screen. If a plate definition is empty (i.e., has not been defined yet), the name stays blank.



There are four softkeys in the **Plate Library** screen:

- **NEW** creates a new plate definition.
- **COPY** duplicates an existing plate definition for easy editing at a later time.
- **DELETE** deletes a plate definition from the plate library.
- **EDIT** modifies an existing plate definition from the plate library.

To exit the **Plate Library** screen without saving changes, press the **MENU** softkey.

Any or all of the plate definition numbers can be used. Selecting the **NEW** softkey automatically assigns the lowest available plate definition number to the new plate definition, as does selecting the **COPY** softkey. Selecting the **EDIT** softkey has no effect if the chosen plate definition is empty.

Creating Plate Definitions

Up to ten different plate definitions can be stored. This makes it easier to set up wash programs and switch between 96- and 384-well plates.

A plate definition is defined by the plate parameters detailed in the table below:

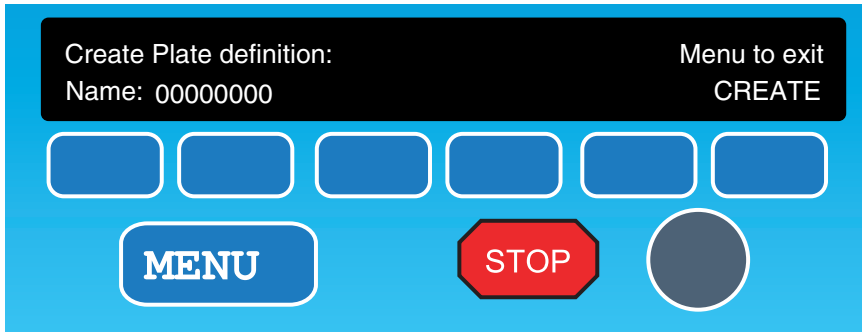
Parameter	Range of Values
Plate Configuration	8X12 or 16X24
CL is the clearance height for Z travel of the wash manifold.	0.0–30.0 mm
HI is the highest probe height for dispensing or aspirating.	0.0–50.0 mm
LO is the lowest probe height for dispensing or aspirating.	0.0 –50.0 mm
$\mathbf{X}_0$ is the X coordinate of Quadrant 1 (or well A1 if using a 96-well plate).	0.0–8.0 mm

Parameter	Range of Values
Y_0 is the Y coordinate of Quadrant 1 (or well A1 if using a 96-well plate).	0.0–8.0 mm
X+ is the X coordinate of Quadrant 4 (for 384-well plates only).	0.0–8.0 mm
Y+ is the Y coordinate of Quadrant 4 (for 384-well plates only).	0.0–8.0 mm
Radius of wells (for 96-well plates only). Used only when wells must be completely drained.	0.0–3.0 mm

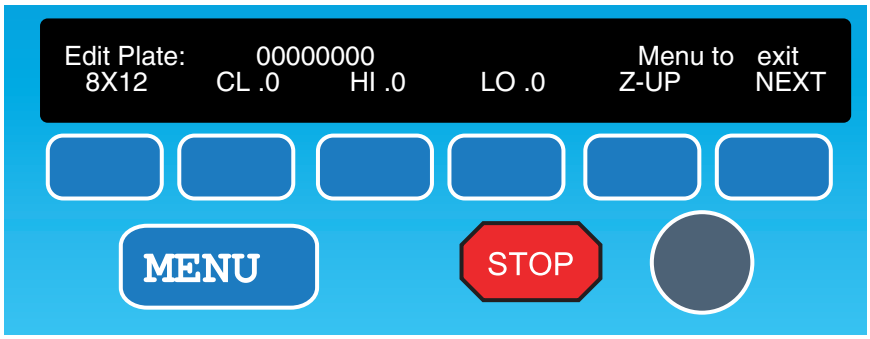
HI and **LO** are user-defined positions in wells in which dispensation and aspiration can be performed. These parameters are set when the plate is defined. The vertical distance between the bottom of the plate and the **HI** or **LO** position determines the residual volume left in the plate after washing. For a cell-based assay, the position at which the cells are washed is important, since dispensing fluid too close to the bottom may dislodge the cells. Dispensing too far from the bottom can provide insufficient washing.

Creating a New Plate Definition

To create a new plate definition:

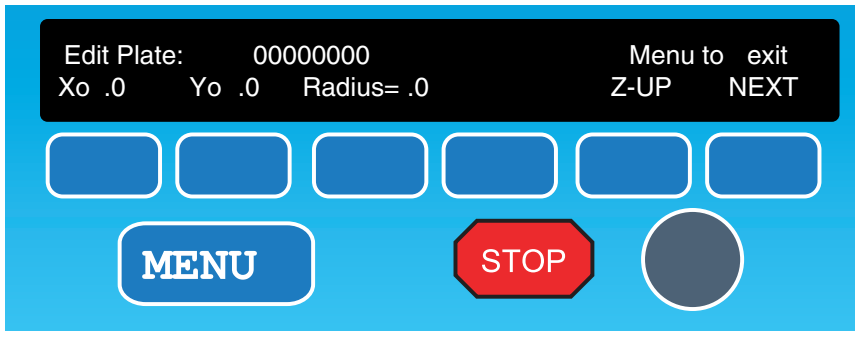
Step	Action
1	Press **PLATES** from the main screen to open the **Plate Library** screen.
2	Press the **NEW** softkey to open the **Create Plate Definition** screen. The software automatically assigns the lowest available plate definition number to the new plate definition. 
3	Enter the name of the new plate definition using the knob to scroll through the letters and numbers. After each character, press the third or fourth softkey to advance to the next character. The name can contain up to 8 alphanumeric characters.

To create a new plate definition: *(continued)*

Step	Action
4	After the name is entered, press the **CREATE** softkey to advance the screen to the **Edit Plate** menu screen. 
5	Place the plate to be defined on the plate carrier. A clear plate (equivalent to the plate used in the assay) should be used so that you can see the probe tips when adjusting their positions inside the wells.

Defining a 96-well Plate

To define a 96-well plate:

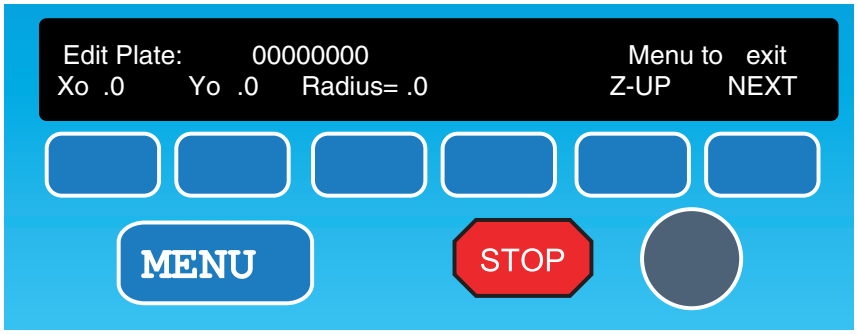
Step	Action
1	Select the **8X12** format by pressing the **Edit Plate** softkey, then using the knob to select the desired plate configuration. **IMPORTANT** You must use the knob to select the 8X12 configuration. If the 8X12 configuration is selected as a default when the **Edit Plate** screen is opened, use the knob to scroll through the plate configurations until the 8X12 configuration appears again and select it.
2	Press the **NEXT** softkey to open the **X/Y Coordinates** screen. Setting the XY coordinates prevents the wash probe manifold from being damaged. 
3	Press the X_0 softkey to set the X coordinate of the wash probe manifold for well A1 of the microplate.
4	Press the Y_0 softkey to set the Y coordinate of the wash probe manifold for well A1 of the microplate.
5	If a circular aspiration is required to completely drain the 96-well plate, press the **Radius** softkey and enter the radius (in mm) around the center of the wells that the wash probes should aspirate. This function allows the washer to completely aspirate the meniscus of liquid at the bottom of the wells.

To define a 96-well plate: *(continued)*

Step	Action
6	Press the **NEXT** softkey to return to the main **Edit Plate** screen.
7	Press the **CL** softkey to set the Z travel height of the wash probe manifold. Use the knob to set the desired height. As the knob is turned, the wash head will move to the corresponding Z travel height.
8	Press the **HI** softkey to set the highest probe position for dispensing or aspirating. The manifold will move to its default position. Turn the knob until the manifold is at the desired position.
9	Press the **LO** softkey to set the lowest probe height for dispensing or aspirating. The manifold usually moves to its default position. Turn the knob until the manifold is at the desired position.

Defining a 384-well Plate

To define a 384-well plate:

Step	Action
1	In the **Edit Plate** screen, select the **16x24** format by pressing the **Edit Plate** softkey, and then use the knob to select the desired plate configuration. **Note** *Note:* Use the knob to set the desired position for the remaining steps in defining a plate definition.The wash probe moves to the corresponding position. Once the desired position is reached, press **NEXT** to store the value.
2	Press the **NEXT** key to open the **X/Y Coordinates** screen. Setting the appropriate XY coordinates prevents the wash probe manifold from being damaged. 
3	Press the X_0 softkey to set the X coordinate of the wash probe manifold for well A1 of the microplate.
4	Press the Y_0 softkey to set the Y coordinate of the wash probe manifold for well A1 of the microplate.
5	Press the **X+** softkey to set the X coordinate of the wash probe manifold for well B2 of the microplate.
6	Press the **Y+** softkey to set the Y coordinate of the wash probe manifold for well B2 of the microplate.
7	Press the **Z-UP** softkey to withdraw the probes and home the Z-axis.

To define a 384-well plate: *(continued)*

Step	Action
8	Press the **X_0** then **Y_0** softkeys to align the probes for the A1 position. **IMPORTANT** Steps 7 and 8 above are critical to prevent damage to the washer and facilitate the adjustments for the **HI** and **LO** positions. **Note** The remaining quadrants will be automatically interpolated by the software.
9	Press the **NEXT** softkey to return to the main **Edit Plate** screen.
10	Press the **CL** softkey to set the Z travel height of the wash probe manifold. Use the knob to set the height approximately 2 mm above the plate. As the knob is turned, the wash head will move to the corresponding Z travel height.
11	Press the **HI** softkey to set the highest probe position for dispensing or aspirating. The manifold usually moves to its default position. Turn the knob until the manifold is at the desired position.
12	Press the **LO** softkey to set the lowest probe height for dispensing or aspirating. The manifold usually moves to its default position. Turn the knob until the manifold is at the desired position. **Note** Confirm that the **LO** and **HI** probe heights do not lift the plate for each coordinate A1 and B2. This ensures the probes will be well-fitted to the plate during operation. Use the **Z-UP** softkey to home the Z axis prior to repositioning the wash head manifold.

Copying a Plate Definition

It may be useful to make minor variations on a plate definition for a different application. Rather than recreating the definition, the **COPY** softkey can be used to duplicate an existing plate definition.

To copy a plate definition:

Step	Action
1	Press the **PLATE** softkey in the main screen.
2	Using the knob, scroll to the desired plate definition to be copied.
3	Press the **COPY** softkey.
4	Enter a new name for the plate definition being duplicated. The name does not have to be unique, but it must be 8 alphanumeric characters.
5	Press the **COPY** softkey again to duplicate the plate definition.
6	The screen automatically advances to the **Edit Plate Definition** screen with the new plate definition displayed. Make modifications to the new plate definition as desired (see sections *"Defining a 96-well Plate"* on page 7-20 and *"Defining a 384-well Plate"* on page 7-21 for details on modifying plate definitions).

Deleting a Plate Definition

In order to remove a plate definition from the plate definitions, the **DELETE** softkey can be pressed in the **Plate Library** screen.

After the **DELETE** softkey has been pressed, turn the knob to scroll to the plate definition to be deleted. Press the **DELETE** softkey again to remove the definition. The plate definition is permanently removed from the plate library, and its number can be used when creating another new or copied plate definition.

Editing a Plate Definition

To edit or view an existing plate definition, select the plate definition from the **Plate Library** screen by using the knob to scroll through the available options. Press the **EDIT** softkey to open the definition, then modify as desired (see sections *"Defining a 96-well Plate"* on page 7-20and *"Defining a 384-well Plate"* on page 7-21 for details on modifying plate definitions).

Creating Wash Programs

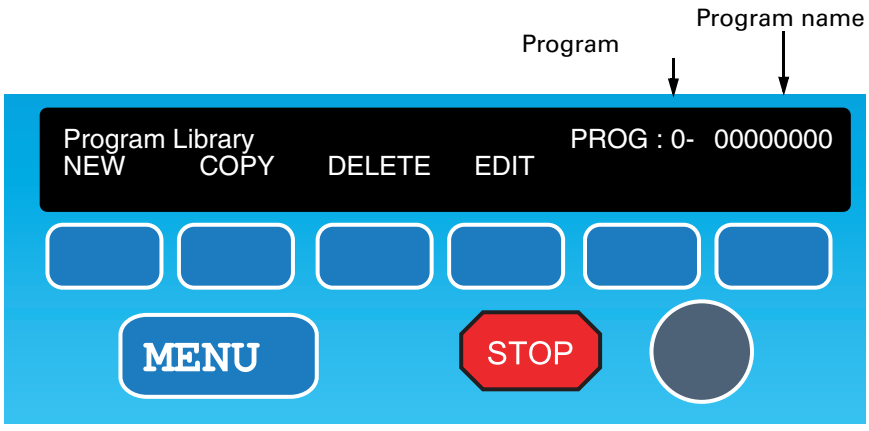
Wash Program Parameters

The plate washer firmware stores up to 40 wash programs. Each program can be customized to meet specific wash requirements. The programmable parameters are listed in the table below.

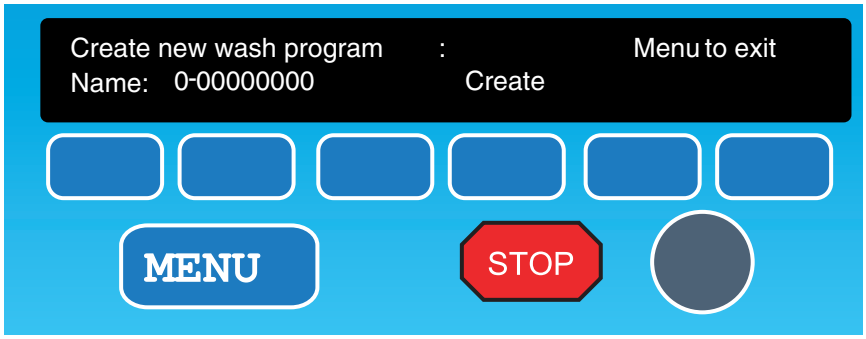
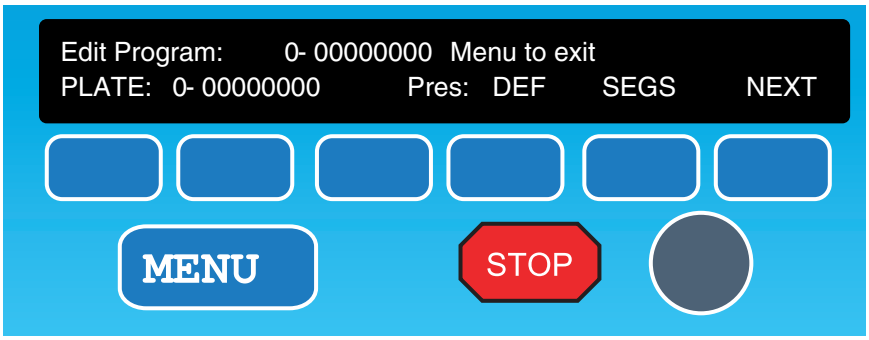
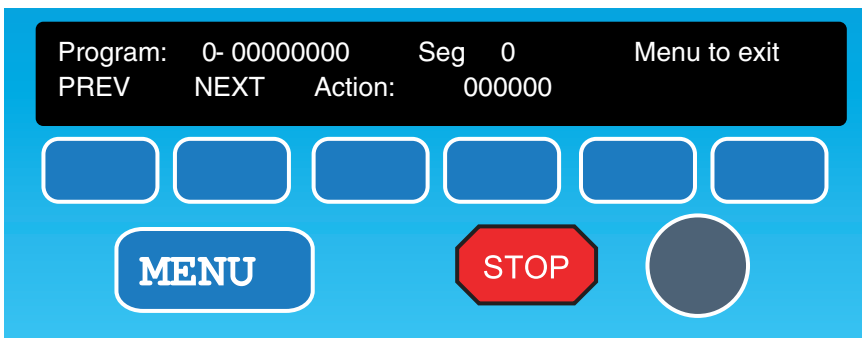
Parameter	Range of Values
Plate Type	Up to ten different plate types are pre-defined. Refer to sections *"Defining a 96-well Plate"* on page 7-20 and *"Defining a 384-well Plate"* on page 7-21 for detailed information on working with plate definitions.
Pressure	**Low**, **Medium**, **High** and **Default**. The pressure values are defined under the **Setup** softkey.
Segments	Up to eight segments per program including: **Aspirate:** removes liquid from the microplate **Dispense:** pipettes liquid into the microplate **Overflow:** simultaneously aspirates and dispenses **Circle:** picks up the fluid meniscus at the bottom of the plate **Soak:** leaves liquid in the microplate **Loop:** repeats segments for repetitive actions **Move:** moves the wash head to a new Z height **End:** terminates the wash program

Creating a New Wash Program

To create a new wash program:

Step	Action
1	Press the **PROG** softkey in the main screen to open the **Program Library** screen. Each program is assigned a number and an alphanumeric name of up to 8 characters. Turning the knob when in the **Program Library** screen scrolls through the plate definitions. The number and name of the chosen program are displayed in the upper right corner of the screen. If a program is empty (*i.e.*, has not been defined yet), the name stays blank. 

To create a new wash program: *(continued)*

Step	Action
2	Press the **NEW** softkey to open the **Create New Wash Program** screen. The software automatically assigns the lowest available program number to the new plate definition. 
3	Enter the name of the wash program to be defined. Use the knob to scroll through the letters and numbers. After each character, press the third or fourth softkey to advance to the next character. The name can have up to 8 alphanumeric characters.
4	After the name is entered, press the **CREATE** softkey to advance the screen to the **Edit Program** screen. 
5	Press the **PLATE** softkey. Use the knob to select the plate type.
6	Press the **PRES** softkey. Use the knob to select the **LOW**, **MED**, **HI** or **DEFAULT** pressure setting.
7	Press the **SEGS** softkey. The display will advance to the action segment programming screen. **Note** The **NEXT** softkey is not used with the FLIPR^{384} system integration. 

To create a new wash program: *(continued)*

Step	Action
8	Press the **Action** softkey until the desired action appears on the display. Program the definable parameters for each action by selecting the softkey beneath it. Use the knob to scroll through the range of values for each parameter:

Segment	Definable Parameters	Range of Values
ASP (aspirate)	Time	1–999 sec
	Probe height	**LO**/**HI**
DSP (dispense)	Volume	10–9990 µL
	Probe height	**LO**/**HI**
FLOW (overflow	Volume	10–9990 µL
	Probe height	**LO**/**HI**
Loop	# of repetitions	1–9999 repetitions
	To segment #	Segments 1–9
CIRC	X times	0–999
MOVE	Probe height	0.0–50.0 mm
END	NA	NA

9	After completing each segment action, press the **NEXT** softkey to store the action and move to the next segment. Up to nine segments can be programmed. It is not necessary to program an **END** segment if all nine segments have been used. **Note** The segment number currently programmed is displayed in the middle of the upper display line. Press the **PREV** softkey to view previously programmed segments or a combination of the **PREV**/**NEXT** softkeys to review a wash program.

Copying an Existing Wash Program

It may be useful to make minor variations on a wash program to use it for a different application. Rather than recreating the program, the **COPY** softkey can be used to duplicate an existing wash program.

To copy an existing wash program:

Step	Action
1	Press the **PROG** softkey in the main screen.
2	Using the knob, scroll to the wash program to be copied.
3	Press the **COPY** softkey.
4	Enter a new name for the wash program being duplicated. The name does not have to be unique, but it must be 8 alphanumeric characters.
5	Press the **COPY** softkey again to duplicate the wash program.
6	The screen automatically advances to the **Edit Wash Program** screen with the new wash program displayed. Make modifications to the new program as desired (see section *"Editing an Existing Wash Program"* on page 7-27 for details on modifying wash programs).

Deleting an Existing Wash Program

In order to remove a wash program from the program library, the **DELETE** softkey can be pressed while in the **Program Library** screen.

After the **DELETE** softkey has been pressed, turn the knob to scroll to the wash program to be deleted. Press the **DELETE** softkey again to remove the wash program. The wash program is permanently removed from the program library, and its number can be used for another new or copied wash program.

Editing an Existing Wash Program

To edit or view an existing wash program, select the wash program from the **Program Library** screen by using the knob to scroll through the available programs. Press the **EDIT** softkey. Edit the program as desired.

Running Wash Programs

Preparing to Run a Wash Program

Plate Washer Checklist

The following is a checklist to ensure that the instrument is ready for use prior to operation:

To prepare to run a wash program:

Step	Action
1	Turn on the plate washer.
2	Check that there is an adequate amount of buffer in the buffer bottle. **Note** Having more than 3.5 liters of wash buffer in the bottle can cause problems with the pressure control.
3	Check all external connections listed below:

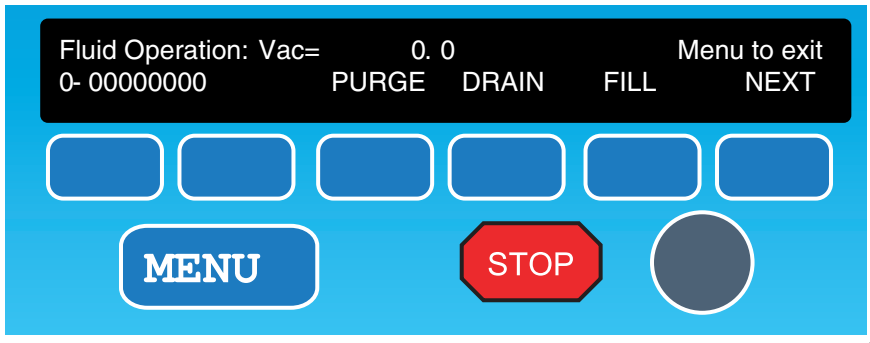
Color	Area	From	To
Yellow	Air	Buffer bottle	Air inlet
Blue	Waste	Aspirate outlet	Waste bottle
Blue	Waste	Waste bottle 1	Waste bottle 2
Green	Vacuum	Waste bottle	Vacuum inlet
Red	Buffer	Buffer bottle	Dispense outlet
NA	Bottle sensor	Buffer bottle	Sensor inlet
NA	Power cord for plate washer	Plate washer rear panel	Electrical wall outlet
NA	Power cord for vacuum	Vacuum power cord	Vacuum extension cord from the plate washer

Step	Action
4	Place an empty plate in the plate carrier.
5	Prime the plate washer (see below).
6	The plate washer is now ready for use.

Priming the Plate Washer

To prime the plate washer:

Step	Action
1	Press the **SETUP** softkey in the main screen.
2	Press the **FLUIDS** softkey to advance to the **Fluid Operations** menu screen.

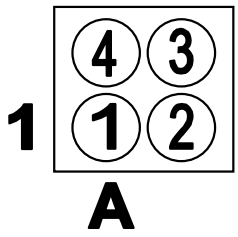


To prime the plate washer: *(continued)*

Step	Action
3	Press the first softkey to choose a plate type.
4	Place an empty microplate of the plate type selected in Step 3 into the plate washer.
5	The following softkeys are used to prime the system: ♦ **Purge** removes air bubbles from the wash manifold. ♦ **Drain** empties system fluid into waste. ♦ **Fill** fills the wash probe manifold with system fluid. It may be necessary to press the desired softkeys several times before the system is primed. When using the **FILL** and **DRAIN** softkeys, the plate washer continues to execute the selected function until the **STOP** softkey is selected.

Running a Wash Program

To run a wash program:

Step	Action
1	Select the wash program to be used. Make sure that the plate type and the wash program are properly defined and compatible with each other.
2	Quadrant specific washing can be selected at the start of any run that uses a 384-well plate. The schematic below illustrates the designations of the individual quadrants. Any combination of the four quadrants can be washed, e.g., 1-1-2, or 2-3, or 1, or 2-3-4, etc. To wash all quadrants, select 1-2-3-4. 
3	Place the plate in the plate carrier.
4	Press the **START** softkey.

Running a Quick Start Wash Program

If the desired wash program requires no editing and priming is not necessary, a quick start can be run immediately from the main screen:

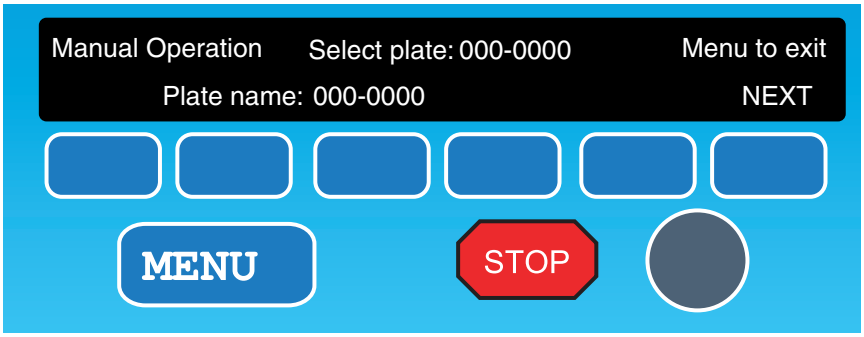
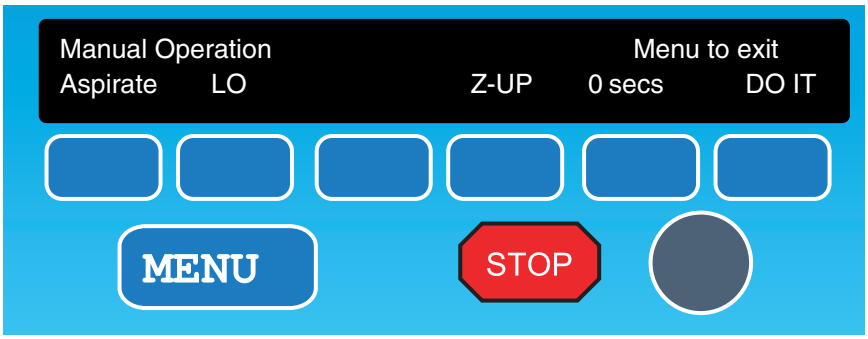
To run a quick start wash program:

Step	Action
1	Select the desired wash program, using the knob to scroll through the available programs.
2	Place the plate to be washed in the plate carrier.
3	Press the **START** softkey. If a valid program has been chosen, the plate washer display changes to the **RUNNING** screen and the wash program starts.

Running the Washer Manually

The plate washer can be controlled manually via the touchpad control panel. The dispense, aspirate and overflow functions can be controlled manually.

To operate the washer manually:

Step	Action
1	Press the **MANUAL** softkey in the main screen to enter the **Manual Operation** screen. 
2	Use the knob to scroll through the plate type library until the desired plate type is displayed on screen.
3	Place the plate in the plate carrier.
4	Press the **NEXT** softkey to advance to the second **Manual Operation** screen. 
5	Select the manual operation to be performed. Refer to the table below for the programmable options for each manual operation.
6	Press the **DO IT** softkey to manually perform the action. **Note** The plate washer will continue to perform the action until the **STOP** key is selected.

Segment	Definable Parameters	Range of Values
ASP (aspirate)	Time	1–999 sec
	Probe height	**LO/HI**
DSP (dispense)	Volume	10–9990 µL
	Probe height	**LO/HI**
FLOW (overflow)	Volume	1-–9990 µL
	Probe height	**LO/HI**

To operate the washer manually: *(continued)*

Step	Action
7	When finished performing manual operations, press the **MENU** softkey to return to the main screen.

Establishing Setup Parameters

Determining the Residual Volume

The following procedure allows you to determine the residual volume in the cell plate.

To determine the plate residual volume:

Step	Action
1	Create a plate definition for the cell plate, including the **X**, **Y**, **Clearance** (**CL**) and **HI** positions by inspection (see section *"Creating Plate Definitions"* on page 7-18 for detailed information). **Note** When defining the **X** and **Y** positions, cycle the head around three times to confirm that all of the parameters are correctly entered. Arbitrarily choose a **LO** position. We recommend at least a 20 µL residual volume for 384-well plates and 80 µL for 96-well plates. The following equation is useful for estimating the **LO** position, but it will not give an exact number: $$LO = T - D + (D/W) \times V$$ T is the observed top of the well in mm, D is the well depth in mm, W is the well volume in µL, and V is the desired residual volume in µL. 11.0 mm is usually an acceptable value for D. For 96-well plates, the suggested W is 360 µL. For 384-well plates, the suggested W is 115 µL.
2	Weigh a dry cell plate.
3	Place a cell plate of the same type as weighed in step 2 on the plate washer. Using manual operation of the plate washer, dispense enough buffer to fill the wells to 50–75% capacity.
4	Using manual operation of the plate washer, aspirate for 3 seconds at the **LO** position.
5	Reweigh the plate. Determine the change in weight of the cell plate.
6	The residual volume (in µL) is determined using the formulas below: For 384-well plates: $$\frac{\text{Change in cell plate}}{\text{Buffer density x 384}} \text{ X } 1000$$ For 96-well plates: $$\frac{\text{Change in cell plate}}{\text{Buffer density x 96}} \text{ X } 1000$$ The buffer density (weight per mL) is determined by weighing 10 mL of buffer, then dividing the weight by 10.
7	To adjust the residual volume, edit the chosen plate type's **LO** value (increasing the **LO** number increases the residual volume, etc.)

To determine the plate residual volume: *(continued)*

Step	Action
8	Repeat steps 3–7 until the desired residual volume is achieved. We recommend at least 20 µL for 384-well plates and 80 µL for 96-well plates. Confirm the results using a fresh plate.

Determining the Maximum Washing Volume

To determine the maximum washing volume:

Step	Action
1	Using manual operation of the plate washer, determine the volume required to dispense buffer to the top of a well already containing the desired residual volume (**DISP VOL**). Avoid over-filling the wells.
2	Use the aspirate command to aspirate the buffer down to the desired residual volume.
3	Repeat steps 1 and 2 until an appropriate maximum wash volume has been determined.

Wash Program Parameters

Initially, we recommend a sequential dispense and aspirate wash program. The following wash parameters are valid for both 384- and 96-well plates (see section *"Creating a New Wash Program"* on page 7-24 for details of creating wash programs):

- **Pressure**: MED
- **Plate**: The desired plate type

Segment	Definable Parameters	Values
1. **ASP**	Time	2 s
	Probe Height	LO
2. **DISP**	Volume	Value determined for Max. Wash Volume
	Probe Height	HI
3. **LOOP**	# of Repetitions	2
	To Segment #	Segment 1
4. **ASP**	Time	2 s
	Probe Height	LO
END		

Setting Up Experimental Parameters

To set up experimental parameters:

Step	Action
1	Prepare a few plates of cells for testing. Load the cells with the appropriate dye mixture.
2	Incubate 1 hour at the optimized temperature.
3	Wash the cells.
4	Run the FLIPR signal test and adjust the signal for a mean of 8,000–12,000 counts. Note any wells in green (low counts). Print the signal test.
5	Run the plate viewer and inspect the wells for consistent signal. Note any holes. Determine if holes are in a certain region or are distributed across the plate.
6	Repeat steps 1 to 5 until the signal test and plate viewer show consistent signals across all wells.

To set up experimental parameters: *(continued)*

Step	Action
7	Add the same volume and concentration of compound to all of the wells. Export the statistics max-min for the first 30 seconds after compound addition (*i.e.* samples 10–30). Calculate the mean, standard deviation and % CV.

Optimizing Wash Conditions

Tips for decreasing the % CV and reducing the holes in the center of the well:

The % CV is Higher Than Desired and the Problem Wells Show a Clear Pattern (All on One Side or in One Corner).

This symptom can be resolved by optimizing the washer settings.

To optimize the washer settings:

Step	Action
1	Raise the **LO** value set for the wash manifold to minimize the risk of aspirating cells off of the plate. **Note** Raising the **LO** value will increase the residual volume.
2	Decrease the aspirate time to minimize the risk of aspirating cells off of the plate.
3	Level the plate's carrier plate on the washer to make the plate carrier and the washer head as parallel as possible.
4	Recalibrate the vacuum to a lower vacuum value. This change may require a longer aspirating time in order to effectively aspirate fluid.

The % CV is Higher Than Desired, and the Problem Wells are Randomly Located Throughout the Plate

This symptom can be resolved by optimizing the FLIPR instrument settings, such as pipettor height during compound dispensation or dispensed compound volume. Call Molecular Devices Technical Support in order to troubleshoot this specific issue.

Maintenance of the Plate Washer

Routine Maintenance

Daily and monthly maintenance should be routinely performed on the plate washer to ensure that the instrument operates reliably and consistently.

Using Soak Mode

During normal operation, salt crystals can build up and clog the valves and tubing. The soak mode keeps the manifold pins in a 96-well plate, containing deionized water, and cycles fluid through the manifold pins at user-defined time intervals. We recommend that you leave the washer in soak mode overnight after each day of use.

To leave the washer in soak mode:

Step	Action
1	Place a 96-well plate with 200 µL /well deionized water on plate carrier.
2	Make sure that the fluid reservoir contains appropriate volume of deionized water.
3	Press the **SOAK** softkey in the main screen, then enter the time interval desired for fluid cycling (*e.g.*, 60 minutes).

Maintenance Schedule

Procedure	Daily	Monthly	As Needed
At the end of the day…place instrument in soak mode overnight or drain dispense buffer from the manifold	x		
At the end of the day…dispose of the waste fluid	x		
At the end of the day…wash bottles and wash manifold with DI water	x		
Wipe down outer surfaces using a cloth dampened with H_20 or alcohol swab.	x		
General machine inspection		x	
Rout out dispense needles with stylus		x	
Decontamination			x
Level the wash probe manifold			x
Blocked probes			x

Cleaning Blocked Probes

Blocked dispense or aspirate probes can be cleared by squirting lukewarm deionized water through the probes with the flushing tool (syringe connector and rubber tubing, supplied with FLIPR^{384}) connected to a syringe or by threading a stylus. (supplied) up into the clogged probe.

System Decontamination Procedure

When to Decontaminate

In the event there is bacterial or fungal growth in the plate washer tubes and valves, it will be necessary to decontaminate the system. The decontamination procedure is also recommended prior to shipment or long-term storage. If the instrument is returned for service or repair, decontamination is mandatory.

System Decontamination Procedure

To decontaminate the washer:

Step	Action
1	Empty all the bottles in the bottle rack. Rinse with DI water.
2	Make up 2 liters of a disinfectant solution. Lysol I. C. is recommended. See below for preparation instructions.
3	Pour equal amounts of the disinfectant solution into each bottle and swirl to rinse the inside of each bottle.
4	Dispose of any fluids in the waste containers and prepare the instrument for operation.
5	Program a 200 µL "overflow wash" that loops 4 times using a standard 96-well microplate. An "overflow wash" is one in which fluid is both added to and aspirated from the microplate wells simultaneously. To program the wash, from the main screen, press **MANUAL →NEXT→ ASPIRATE→DISPENSE→OVERFLOW**.
6	Place an empty 96-well microplate in the plate carrier.
7	Run the program created in step 5. It is recommended that the procedure be repeated using distilled water to remove residual disinfectant.
8	Once the procedure is complete, empty all of the bottles and air dry them.
9	The system is now ready for shipment or long-term storage.

Preparation of Lysol I. C

Lysol I.C. is an instrument disinfectant presoak. It was chosen for decontamination of liquid handling systems because it has the same decontamination properties as Professional Lysol Foam Cleaner. Currently, Lysol I.C. is concentrated, has a red tint, and needs to be diluted. Previously, Lysol I.C.was pre-diluted and had a blue-green tint. Dilute the Lysol I.C. at a 1:200 ratio.

Note Please call Technical Support for information regarding internal decontamination.

Incompatible Wash Fluids

Bleach, DMSO, highly concentrated acids and trichloroacetic acid (TCA) are not compatible with the plate washer.

! WARNING ! Never use bleach to clean or flush your system. Bleach can damage the plate washer's deck, valves, sampling tips and adapters. Bleach leaches ions out of stainless steel which causes pitting and rusting.

Shipping the Plate Washer

About Shipping

If the plate washer needs to be returned for service or transported to another location, it must be repackaged in the original packing materials. Other forms of commercially available packing materials are not recommended, and their use can void the washer's warranty. If the original packing materials have been damaged or lost, contact Molecular Devices Service Department for replacement packing.

Before you ship the instrument for repair:

- Contact the Molecular Devices Service Department to arrange for shipment and obtain a Return Authorization Number (RAN). The RAN must appear on the outside of the shipping box and should be referenced in the shipping documents.
- Instruments returned to the manufacturer for repair require a Certificate of Decontamination. Decontaminate the instrument as detailed in section *"System Decontamination Procedure*" on page 7-37. Photocopy the Certificate of Decontamination located in Appendix F, *"Decontamination Certificate"* and fill it out completely. Include the certificate with all appropriate documentation.

Base Unit Packing Instructions

To ensure proper and safe transit of the plate washer, the XY axis motion control must be immobilized to prevent damage.

To pack the base unit for shipping:

Step	Action
1	Remove the plate carrier, the tubing and vacuum pump connections from the rear panel of the plate washer base unit. DO NOT TURN THE POWER OFF.
2	Position the wash head for transit by selecting the following softkeys in the order presented, starting from the main screen: **SETUP**→**INIT**→**SHIP**→ **RECALL**. The wash head will move into position in preparation for shipping.
3	Turn the plate washer on its side, exposing the bottom panel. Insert the locking pin in the hole marked "Locking Pin". Ensure that the pin is inserted up to the base of the ring.
4	Unplug and remove the power cord from the base unit.
5	Fit the blue foam insert around both of the long sides of the base unit.
6	Insert the two rectangular foam pieces on either side of the wash head. Ensure that each of the rectangular foam pieces crosses both of the side foam pieces.
7	Lift the plate washer from the bottom of the unit and place it in the original box with its accessories. Secure the box.

Bottle Rack Packing Instructions

If the bottle rack is to be shipped or returned, it should undergo the decontamination procedure before shipping to ensure that no fungal or bacterial growth occurs during transit.

To pack the bottle rack, if returning:

Step	Action
1	Remove all tubing connections from all bottles in the bottle rack.

To pack the bottle rack, if returning: *(continued)*

Step	Action
2	Empty any remaining fluid from all bottles. Cap the bottles.
3	Place the bottles and rack in the original packing box.
4	First fit the larger cutouts in the cardboard bottle insert around the bottles, then fold the insert over to fit the smaller cutouts around the bottles.
5	Reconnect the tubing or neatly place the tubing around the bottles.
6	Secure the box for shipment.

Troubleshooting and Validating the Plate Washer

Troubleshooting

If the plate washer is experiencing problems, careful observation will aid in correcting the problem. Listed below are some common problems and probable causes

Observation	Possible Cause	Recommended Action
Excessive residual fluid after aspiration (evenly distributed)	Not enough vacuum	Check the vacuum. It should be 15 in Hg @2 CFM.
	Wash manifold is too high for the plate	Adjust the height of the manifold.
Excessive residual fluid after aspiration (unevenly distributed)	Wash probe manifold is not level	Level the wash manifold.
Fluid being removed during soak operation	Valves are leaking	Clean/replace valves
	Vent filter is dirty	Clean/replace vent filter
Dispensing is uneven	Not enough pressure	Check the pressure.
	Large air bubbles in the wash dispense head	
	Dispense probe out of position	
1 or more wells are not filling	Probe is clogged	
Wash head is dripping	Insufficient vacuum	Check the vacuum. It should be 15 in Hg @2 CFM.
	Air leak through the manifold or valves.	

Also, see section *"Establishing Setup Parameters"* on page 7-32 for additional information on configuring the plate washer correctly.

Validating the Washer

Prior to shipment, the instrument is validated at the factory. To validate additional wash head manifolds or to verify performance of the wash head for quality control purposes, refer to the procedure below.

To validate the washer:

Step	Action
1	Make up the following solution in a dispense buffer bottle and mix well: ♦ 1.2 mL IRIS egg yellow dye in 1 liter H_20 (alternatively: 0.1% green food coloring dye, 1 mL per 1 liter H_20) ♦ 1 drop Tween® 20
2	Ensure that an appropriate plate type and Fx have been established.
3	Press the **MANUAL** softkey in the main screen.
4	Use the knob to scroll through the Plate Type Library. Select the plate type to use for the validation procedure.
5	Press the **ASPIRATE** softkey until it displays **DISPENSE**.

To validate the washer: *(continued)*

Step	Action
6	Program the **DISPENSE** function parameters: ♦ Height **LO**/**HI** ♦ Volume (µL): choose a typical wash delivery volume, e.g. 200 µL
7	Press the **DO IT** softkey. The plate washer will dispense the programmed volume.
8	Press the **Z-UP** softkey and remove the plate from the plate carrier.
9	Place the plate in a microplate reader programmed with the following parameters: (shake for 30 seconds before reading to remove bubbles and make the menisci more uniform) primary wavelength 405 nm, secondary wavelength 620 nm, endpoint read.
10	Calculate the mean, SD, and % CV of the optical densities for the entire plate, each row and each column. Specifications are as follows: ♦ Each column < 10% CV ♦ Each row < 10% CV ♦ Entire plate < 8% CV

8 Maintenance

Introduction

In This Chapter

This chapter provides procedures for the following:

Performing the Yellow Plate Test

About the Yellow Plate Test

The Yellow Plate Test must be performed once a day, and the printouts should be kept in a Maintenance folder. The Yellow Plate Test should be run every day under the same conditions so that results can be compared over time.

Note Make sure that the instrument is warmed up before running a Yellow Plate Test or an assay.

CAUTION Store your yellow plate in a safe place, away from bright light on an even surface. Avoid scratching the bottom of the plate, since this could affect the coefficient of variation.

Yellow Plate Test Parameters for a 384-well Plate

Set the following parameters for a 384-well yellow plate:

- Install the ledge support in the cell plate position (no mask).
- Laser power at 600 mW. Adjust with the **UP** and **DOWN** arrows on the remote control pad.
- Make sure that the laser is set in **Light** mode.
- Set the camera f/stop to f/2. Check by looking at the camera lens through the filter door.
- Set the exposure time to 0.05 second, located in the **General** tab of the **Experiment Setup** dialog box (see Chapter 4 *"Software Installation and Description"* for details).
- For FLIPR^3, set the camera gain to 100.

Yellow Plate Test Parameters for a 96-well Plate

Set the following parameters for a 96-well yellow plate:

- Install the slit-shape mask in cell plate position.
- Laser power at 600 mW. Adjust with the **UP** and **DOWN** arrows on the remote control pad.
- Make sure that the laser is set in **Light** mode.
- Set the camera f/stop to f/2. Check by looking at the camera lens through the filter door.
- Set the exposure time to 0.05 second, located on the **General** tab of the **Experiment Setup** dialog box (see Chapter 4 *"Software Installation and Description"* for details).
- For FLIPR^3, set the camera gain to 100.

Yellow Plate Test Procedure (384- or 96-well Plate)

To perform the Yellow Plate Test:

Step	Action
1	Place the yellow plate on the stage in the cell plate position.
2	Set the parameters as indicated in the previous section.
3	Take a picture by selecting **Signal Test** from the **Run** menu. Acceptable yellow plate results are a standard deviation (less than 3.5% and fluorescence counts between 10,000 and 20,000). If the test results are outside of the range of acceptable values, perform the optical correction protocol (see Appendix E, *"Optical Calibration Protocol,"* for details).

To perform the Yellow Plate Test: *(continued)*

Step	Action
4	Print the results and keep them in a “Maintenance” folder by the instrument to track the instrument performance over time.

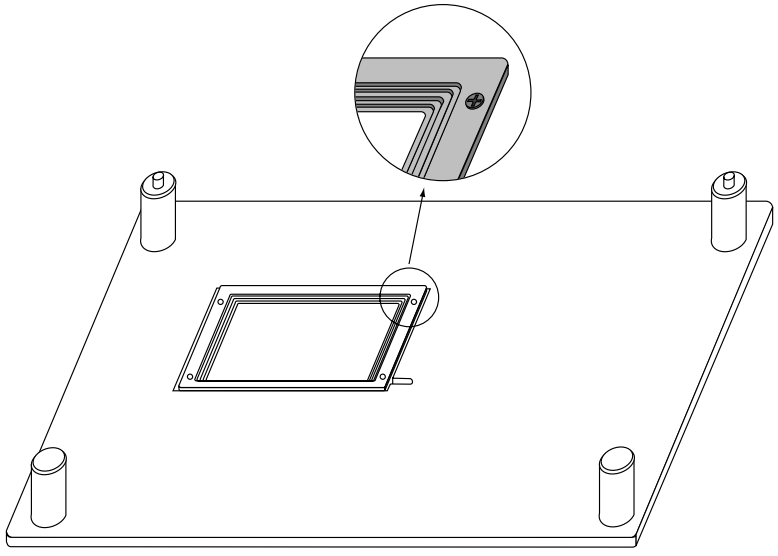
Changing the Plate Mask or Ledge

About the Plate Mask and Ledge

The plate mask is used with 96-well plates, while the ledge is used with 384-well plates. When using a 96-well plate in a "round-mask" mode, the entire well is read as opposed to the center slit. The plate mask and plate ledge are fastened to the bottom of the stage over the cell plate position (Position 1).

Changing the Mask or Ledge

To change the mask or ledge:

Step	Action
1	Remove the stage from the FLIPR platform.
2	Turn the platform upside down.
3	Use a Phillips-head screwdriver to remove the four screws that fasten the current ledge or mask to the stage, and then use the same screws to fasten the desired mask or ledge to the stage. **IMPORTANT** The plate ledge must be installed with its smooth side against the bottom of the stage. **Note** The diagram below shows the underside of the stage when the ledge is installed. Note that the concentric "steps" on the ledge are installed facing away from the underside of the stage. 

Laser and Camera Adjustments

Laser Mirror Alignment (Optional)

If calibration and alignments did not pass, you may have to align the laser mirror. Perform the laser mirror alignment procedure each time the Yellow Plate Test generates standard deviations that are higher than the acceptable limit. The laser alignment procedure consists of optimizing the position of the laser mirrors so that the maximum power (= light) output is obtained for a set current (= Amp) input. Usually, the mirror's position becomes misaligned when the positions of the two silver knobs, located on the back side of the laser, have unintentionally been modified.

Pull the laser remote control close to the back of the laser next to the computer monitor. Press the **Peak** key on the remote control. This function automatically sets the laser to **Current** mode. The laser is set to a given current, and the wattage is variable.

To align the laser mirror:

Step	Action
1	Touch the top silver knob and slowly rotate it clockwise while watching the remote control monitor. Your goal is to position the silver knob so that the wattage is maximal.
2	If the wattage increases, very gently keep rotating the knob in the same direction until you reach the maximal value. If you rotate beyond the optimal position, the wattage values will start dropping.
3	If the wattage values initially decrease, rotate the silver knob counter-clockwise until you reach the maximal wattage.
4	Repeat steps 1–3 with the lower silver knob.
5	In order to check the effectiveness of the laser alignment procedure, perform a Yellow Plate Test. If the standard deviation is not decreased, perform the optical correction protocol (see Appendix E, *"Optical Calibration Protocol,"* for details). **! WARNING ! Never touch both silver knobs at the same time.**

Maintaining the Laser Cooling System

Maintain an appropriate cooling system for your laser. Make sure that the laser cooling system is always turned on prior to turning on the laser remote control and that the cooling system stays on for at least 5 minutes after the laser has been shut down. Refer to your Pre-Installation Manual for specific temperature ranges. If the cooling system reaches temperatures out of the acceptable range, the laser will automatically shut itself off, even in the middle of an experiment!

Maintaining Proper Air Flow

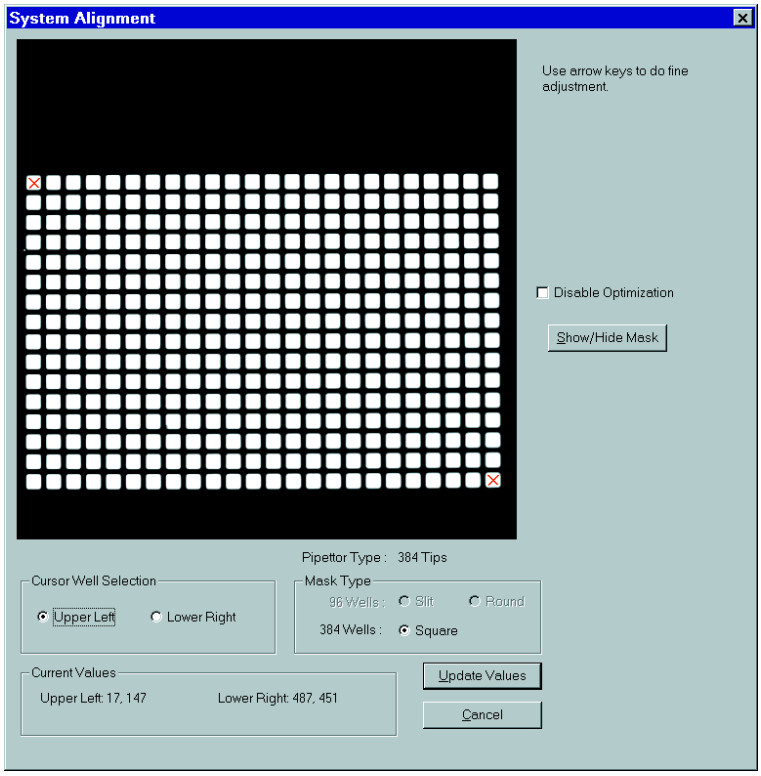
Maintain a reliable air source of 90–100 psi. The pipette tip sealing system and the heated stage lids use air and will not function properly unless the air pressure is sufficient. Insufficient air pressure will activate either of the following messages:

- "Air supply is off! "(if you are in start-up check) or
- "Unable to activate pneumatic cylinder in experiment" (in other modes)

to appear in the Interlock section of the software's FLIPR Status Bar, and the experiment will be halted.

Maintaining Camera Alignment

The FLIPR system computes the fluorescence signal differently, depending on the type of plate (96- or 384-well plate). In the case of the 96-well plate, it depends on whether the mask is slit-shape or in round-shape mode. Therefore, for accurate results, it is critical that you specify these parameters early on in the experimental setup. The **System Alignment** dialog box (Figure 6-2, accessed by choosing **Alignment** from the **Setup** menu) allows you to select the type of cell plate used and the type of mask. Furthermore, you may have to perform the camera alignment procedure if the standard deviation of the Yellow Plate Test increases above the acceptable limit. This will insure that the software and the camera are aligned.



Even if the cell plate and/or mask are no different than for the previous assay, the alignment function can be used to optimize the alignment of the camera picture with the well positions, potentially decreasing the variability across the plate. Although the cell plate and the mask can vary, the alignment procedure is the same for 96- and 384-well formats.

Note The laser has to be on and warmed up in order to perform the alignment procedure.

Note Use the yellow plate matching the cell plate that will be analyzed (384- or 96-well).

Note In the case of the 96-well plate, ensure that the desired mask is in place (see above for mask placement instructions).

To align the camera:

Step	Action
1	The **Cursor Well Selection** panel of **System Alignment** dialog box contains two buttons: **Upper Left** and **Lower Right.** First, select the **Upper Left** button, then click the cursor in the exact center of the well in the upper left corner. A pink **X** will be placed in the well. You can use the arrow keys on the keyboard to adjust the position of the **X**. Next, select the **Lower Right** button in the dialog box, click the cursor in the exact center of the lower right well and use the arrow keys to adjust the position of the **X**.
2	Select the type of plate and mask from the options in the **Mask Type** panel.
3	Check that the **Disable Optimization** button is checked or unchecked, as you prefer. When unchecked, the alignment of the wells with the camera will automatically be optimized. When the button is checked, the optimization function will be disabled, and the alignment is set exactly as you clicked in the upper left and lower right wells. Most users keep this box unchecked to optimize alignment.
4	Click the **Show/Hide Mask** button. This displays the current alignment and allows you to determine whether or not further optimization is required.
5	To set the new alignment parameters, click the **Update Values** button to implement the new changes and close the dialog box.

Laser Argon-ion Recharging

Every 100 hours, the Argon-ion laser must be recharged. To do so, simply press the **UP** arrow on the laser remote control until the Amp value is approximately 30 Amp. Leave the laser at this high setting for approximately 30 minutes. This will free some Argon from a reservoir located in the front of the laser. This procedure can be performed during the warm-up time of the system.

! WARNING ! If the laser is not recharged regularly, the Argon pressure can become low and the laser may automatically shut itself off, even in the middle of an experiment. If this happens, the "Low pressure" error message will be displayed in the laser remote control's monitor. If the error message appears, turn the laser back on and recharge it.

Computer Backup

As soon as you start using your FLIPR system, make a backup copy of your **Setup.flp** file. This file contains all the pertinent information for your instrument, such as the pipettor position.

Although the computer provided with your FLIPR system has a large hard drive, the FLIPR files are also large (particularly if you save data in the fid file format). Make sure the data files are backed up and removed from the hard disk on a regular basis.

9 Troubleshooting

Introduction

In This Chapter

This chapter contains tables of symptoms and solutions to help you diagnose and repair problems with the FLIPR system. In some cases, you will need to contact Molecular Devices' Technical Support group: (800) 635-5577.

Troubleshooting FLIPR System

Troubleshooting Start-up

Symptom or Error Message	Possible Causes	Solutions
"FLIPR is unable to establish a communication link to the Anafaze heater control. Do you want to continue without hardware support?"	FLIPR system components were powered on in wrong sequence.	Restart system, being careful to follow the sequence given in Chapter 3, *"System Start-up and Shut-down Procedures."*
	Loose cable connections.	Recheck connections. If none are loose, contact Technical Support.
"The specified AD converter is not installed and the software will not communicate with FLIPR."	CCD camera is powered off.	Restart system (see Chapter 3, *"System Start-up and Shut-down Procedures."*)
	CCD camera or controller failure.	Contact Technical Support.
"Please turn on 80 psi air supply."	Air supply is not on, or air pressure is too low.	Confirm air supply is on (90 psi recommended).
	Air regulator failure.	Contact Technical Support.
"Main door is not fully opened, please check it and click **Retry**."	Interlock problem.	Check that the door cable stops are in place on each side of the door. This error must be cleared by clicking **Retry** and not **Cancel**.
FLIPR start screen appears with hourglass and does not proceed.	Camera/controller/ card problem.	Restart system (see Chapter 3, *"System Start-up and Shut-down Procedures."*) Contact Technical Support.
Software immediately opens without communicating with FLIPR and Main Screen's System Setup panel shows "No hardware setup."	Setup.flp file is set to open software without communicating with FLIPR.	Close FLIPR software. Open the C:/FLIPR/BIN/setup.flp file. Change HARDWARE=0 to HARDWARE=1, save and close the file, then relaunch the FLIPR software.
The stacker plate carrier strikes the FLIPR main door.	Stacker initializing sequence conflicts with closed FLIPR door.	Turn on air supply, power off FLIPR, power on stacker, wait for stacker to initialize, power on FLIPR, then launch FLIPR software.
"FLIPR indicates the Big Cylinder is NOT functioning. Please call Technical Support."	Pipettor position problem.	Power off FLIPR, manually move pipettor over drug position 2, then restart system.
	Interlock problem.	Contact Technical Support.
"Pipettor Hit Home" appears in Main Screen's **Interlock** panel and experiment stops.	Pipettor position problem.	Contact Technical Support.

Symptom or Error Message	Possible Causes	Solutions
"This control file does not require the heated stage. The current setup indicates a heated stage is installed."	**fcf** file incorrectly configured.	Select **Hardware** from the **Setup** menu, then choose the correct stage.

Troubleshooting the Laser

Symptom or Error Message	Possible Causes	Solutions
"0 Watts" displayed on the laser remote control module.	Laser mirrors are misaligned.	Contact Technical Support.
Laser starts working, then shuts off followed by the "Laser Off" message on the laser remote control module and a second error message.	Varies.	Check Coherent Operator's Manual for the second error message; contact Technical Support.
"Low water flow."	Decrease in cooling water volume.	Check water supply volume (both loops.)
	Clogged filters.	Replace filters as needed.
"Inlet Water Temp."	Cooling fluid temperature above or below specifications.	Repair cooling system to deliver fluid at the correct temperature. Contact Technical Support.
"Auto fill, 96 h until shutdown."	Laser requires recharging.	Press exit on remote. Place in CUR mode, ramp up to 40A for 30 min. to 1h.
"Low Pressure."	Laser requires recharging.	Start laser at 40 A current and run system until "Autofill OK" appears on laser remote module.

Troubleshooting the Yellow Plate Test

Symptom or Error Message	Possible Causes	Solutions
Signal test mean is above the expected range (10,000-20,000). The message "saturation detected, data may be invalid" may appear in the signal test panel.	Incorrect settings.	Set laser to 600 mW, the exposure time to 0.05 sec and the f/Stop to 2.
	Incorrect filter choice.	Select **Experiment** from the **Setup** menu, then click the **General** tab. Choose filter #1.
		Check that the 510–570 nm bandpass filter is in place in filter position number 1.
Alternating dark and light rows of wells seen in plate viewer.	Laser shutter not opening correctly.	Contact Technical Support.

Symptom or Error Message	Possible Causes	Solutions
Wells are cut off in plate viewer.	Change in the alignment settings.	Perform Optical Correction Protocol (see Appendix E, *"Optical Calibration Protocol."*) Contact Technical Support.
	Dirty/misaligned optics.	Contact Technical Support.
Fluorescent counts are about 500.	Laser is powered off.	Power on laser.
	Laser shutter closed.	Contact Technical Support.
	Camera failure.	Contact Technical Support.
Standard deviation for the signal test is greater than 3.5% and fluorescent counts may or may not be in the correct range.	Laser not fully warmed up.	Wait until laser has run for at least 30 minutes.
	Camera not fully cooled down.	Wait until CCD camera has cooled for at least 30 minutes.
	Optical correction out of specifications.	Perform Optical Correction Protocol (see Appendix E, *"Optical Calibration Protocol."*)
	Stage was changed or moved.	Perform Optical Correction Protocol (see Appendix E, *"Optical Calibration Protocol."*)
	Laser mirrors misaligned.	Align laser mirrors. If the problem persists, contact Technical Support.
	Optics are dirty or failing.	Contact Technical Support.
	Calibration plate failure — plate is dirty or scratched.	Clean or replace calibration plate with lens paper. DO NOT use lab wipes.

Troubleshooting the Pipettor

Symptom or Error Message	Possible Causes	Solutions
Pipettor strikes the metal tip carrier and experiment stops.	Pipettor position failure.	Power off and restart system. Check tip carrier hooks. If they are bent up or down, replace the tip carrier. Test load tips to be sure pipettor is still correctly aligned. Contact Technical Support.
Tips are not loaded when commanded.	Carrier hooks are bent.	Replace tip carrier. Contact Technical Support.
	Check pipettor alignment.	Contact Technical Support.
Tip box was moved while tips were on pipettor.		Choose **Run→Special Operations →Unload Tips** (unload tips to plate position 4).

Symptom or Error Message	Possible Causes	Solutions
Experiment stops and the pipettor does not respond to commands. Message appears: "FLIPR indicates the Big Cylinder is NOT functioning. Please call Technical Support."	Pipettor position failure.	Power off FLIPR, manually move pipettor over drug position 2, then restart FLIPR.
	Interlock problem.	Contact Technical Support.
System restarted with tips on pipettor.		Choose **Run→Special Operations→Unload Tips** (unload tips to plate position 4).
"Pipettor Hit Home" error displayed in Main Screen's Interlock panel; experiment stops.	Pipettor position failure.	Contact Technical Support
When the main door opens the tip box is attached to tips (failure occurs during pipettor movement toward compound plate).	Tip box failure.	Remove tip box from tips. Choose **Run→Special Operations →Unload Tips** (unload tips to plate position 4). Contact Technical Support.
When the main door opens, the tip carrier is attached to pipettor; pipettor is frozen (failure occurs soon after the start of the experiment).	Pipettor calibration failure.	Contact Technical Support.
Pipettor strikes heated stage clamshell lid.	Air pressure problem on heated stage.	Contact Technical Support.
"Air supply is OFF!" appears in interlock Main Screen's Interlock panel; experiment stops during tip unloading.	Air pressure is set too low.	Contact Technical Support. Leave tips on pipettor until the end of the experiment.
Some tips are left on pipettor after unloading.	Pipettor calibration failure.	Contact Technical Support.

Troubleshooting the Tip Washer

Symptom or Error Message	Possible Causes	Solutions
Tips are not washed.	Inlet tubing is not primed.	Choose **Run→Special Operations → Tip Washer Prime**.
	Wash basin is not filled.	Check for errors (low washing fluid, high waste, peristaltic pump is moving fluid, vacuum pump is powered on).
	Pipettor is not set low enough into the wash basin.	Contact Technical Support.
	Software protocol is not set correctly.	Choose **Setup →Experiment** then click the **Tip Washer** tab and adjust settings.
	Washing buffer ineffective in cleaning tips.	Change buffer formula. Contact Technical Support.
	Valve on reservoir bottle is stuck closed.	Recheck all blue connections and listen for the valve to open and close.
Wash fluid does not drain to waste bottle.	Vacuum leak in waste bottle.	Check all connections.
	Vacuum pump is powered off.	Power on vacuum pump. Contact Technical Support.
Tip washing is halted and the message appears "Liquid level in one of the washer tanks has reached its limit. Please check the tanks and restart."	Waste bottle is full.	Empty waste bottles, refill wash bottle.
	Electrical connection failure.	Recheck red connections. Contact Technical Support.
Tip washing is halted and the message appears "FLIPR is unable to initialize washer pump. Please check the pump and restart."	Peristaltic pump is powered off.	Power pump on. When on, the pump screen should read "0". At initialization, the screen should read "PO1". Initialization can be run by choosing **Run→Special Operations→Tip Washer Prime.**
	Electrical connection failure.	Contact Technical Support.
Activate tip washer is greyed out in **Automation** tab of **Experiment Setup** dialog.	Hardware settings are incorrect.	Select **Hardware** from the **Setup** menu, then select the **Tip washer installed** button.

Troubleshooting Data

Symptom or Error Message	Possible Causes	Solutions
Results are negative or not optimized.	Hardware has not been optimally calibrated for the assay being run.	See "Optimizing an Assay" on page 5-33.
	Biofilms growing in wash tubing.	Change tubing, put clean bottles in tip washer and plate washer.
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. Use MDC Mix and Read Reagents.
Statistical results do not conform to the expected results.	Data analysis has not been optimally set up.	Refer to Appendix C, *"FLIPR Systems Data Processing Algorithms."*
Data parameters need to be reset (subtract bias, spatial uniform correction, positive control, negative correction factor) for each experiment.	**fcf** file parameters not set up correctly.	Choose **Setup→ Defaults→ Graph Setup** and **Control Well Setup**, adjusting parameters as necessary.
False negative wells.	Pipettor adjustment problem.	See "Optimizing an Assay" on page 5-33.
	Tip problem.	Contact Technical Support.

General Troubleshooting

Symptom or Error Message	Possible Causes	Solutions
Data files cannot be found.		Use Windows' Find utility to search for file names and determine where files were saved.
Hard drive fills up with data files.	Files unnecessarily saved in **FID** format.	Open **FID** files, save as **FWD** files and discard **FID** files (note: **FWD** files cannot be re-saved as **FID** files).
	Data not archived	Clean off hard drive.
FID Viewer... option on the **Run** menu is grayed out.	File saved in **FWD** format	Save subsequent data as **FID** files.
The plate washer is not activated.	**Hardware** settings are not correct.	Choose **Hardware** from the **Setup** menu, then click the **Plate washer installed** button.

Troubleshooting the Stacker

Symptom or Error Message	Possible Causes	Solutions
Stacker stage does not move plate into position.	Various possible causes.	Contact Technical Support.

Symptom or Error Message	Possible Causes	Solutions
Stacker moves cell plate too quickly or slowly and the wells are splashed	Stacker stage requires calibration.	Contact Technical Support.
Bar code reader misreads the bar code and filename reads "Badbarcode."	Misplacement of bar code or incorrect type of barcode.	Bar code must be on long side of plate. Place label as low as possible. Use labels with numbers on top.

Troubleshooting the Camera

Symptom or Error Message	Possible Causes	Solutions
All wells show artificial spikes at the same time.	Camera shutter problem.	Contact Technical Support.
Some wells show artificial spikes at random times.	CCD camera or controller problem.	Contact Technical Support.

Consumables Used With the FLIPR System

A

Introduction

About This Appendix

This appendix contains a catalog of all the consumables that are used with the FLIPR system.

Plates

96-Well Black Wall Plates

96-Well Black Wall Plates	Suggested Supplier	Phone Number	Item #
Black wall plates, clear bottom, tissue culture treated, sterile, 96-well	Becton Dickinson	800-343-2035	353948
	Corning/Costar	800-492-1110	3603
	Greiner (distributed by E&K)	408-378-2013	781091
	Greiner without lids (distributed by E&K)	408-378-2013	781092
Black wall plates, clear bottom, tissue culture treated, sterile, 384-well, *poly-D-lysine coated*	Becton Dickinson	800-343-2035	356640
Black wall plates, clear bottom, tissue culture treated, sterile, 384-well, *collagen coated*	Becton Dickinson	800-343-2035	356649

96-Well Compound Plates

96-well Compound Plates	Suggested Supplier	Phone Number	Item #
V-bottom plate, 96-well	Becton Dickinson	800-343-2035	353263
	Corning/Costar	800-492-1110	3363
	Greiner (distributed by E&K)	408-378-2013	651101 polystyrene
	Greiner (distributed by E&K)	408-378-2013	651201 polypropylene
	Nalge/Nunc	800-766-7000	249128 polystyrene
U-bottomed plate, 96-well	Greiner (distributed by E&K)	408-378-2013	650101 polystyrene
	Greiner (distributed by E&K)	408-378-2013	650201 polypropylene

384-Well Black Wall Plates

384-well Black Wall Plates	Suggested Supplier	Phone Number	Item #
Black wall plates, clear bottom, tissue culture treated, sterile, 384-well	Becton Dickinson	800-343-2035	353263
	Corning/Costar	800-492-1110	3712
	Packard Instrument	800-856-0734 203-639-2404	6005261
	Greiner with lids (distributed by E&K)	408-378-2013	781091
	Nalge/Nunc	800-766-7000	142761
Black wall plates, clear bottom, tissue culture treated, sterile, 384-well, *poly-D-lysine coated*	Becton Dickinson	800-343-2035	356663
Black wall plates, clear bottom, tissue culture treated, sterile, 384-well, *collagen coated*	Becton Dickinson	800-343-2035	356667

384-Well Compound Plates

384-well Compound Plates	Suggested Supplier	Phone Number	Item #
Flat bottom plate, 384-well	Corning/Costar	800-492-1110	3702 polystyrene w/lid
	Greiner (distributed by E&K)	408-378-2013	781101 polystyrene w/lid
	Becton Dickinson	800-343-2035	353265 polypropylene
	Greiner (distributed by E&K)	408-378-2013	781201 polypropylene w/lid

Pipetting Accessories

Pipetting Consumables

Item	Suggested Supplier	Phone Number	Item #
FLIPR pipette tips, black, non-sterile, 96-well, 250 µL, type A	Molecular Devices	800-635-5577 408-747-1700	9000-0549
FLIPR pipette tips, clear (for pipettor calibration), 96-well, 250 µL, type A	Molecular Devices	800-635-5577 408-747-1700	9000-0548
FLIPR pipette tips, black, non-sterile, 96-well, 250 µL, type B	Molecular Devices	800-635-5577 408-747-1700	9000-0618
FLIPR pipette tips, clear (for pipettor calibration), non-sterile, 96-well, 250 µL, type B	Molecular Devices	800-635-5577 408-747-1700	9000-0617
Alternative tips for FLIPR^{384} 96-well format: Autotips, black, non-sterile, 96-well, 200 µL	Robbins Scientific	800-752-8585 408-734-8500	1043-24-5
Alternative tips for FLIPR I: Autotips, black, non-sterile, 96-well, 200 µL	Robbins Scientific	800-752-8585 408-734-8500	1043-24-O
Alternative clear tips for FLIPR I (for pipettor calibration): Autotips, clear, non-sterile, 200 µL	Robbins Scientific	800-752-8585 408-734-8500	1043-04-0
For FLIPR^{384} quadrants: Micro Autotips black, non-sterile, 96-well, 50 µL	Robbins Scientific	800-752-8585 408-734-8500	1043-26-5
FLIPR pipette tips, clear, 384-well, type A (fits on a 384-head, type A)	Molecular Devices	800-635-5577 408-747-1700	9000-0257
FLIPR pipette tips, clear, 384-well, type B (fits on a 384-head, type B)	Molecular Devices	800-635-5577 408-747-1700	9000-0512
FLIPR pipette tips, black, 384-well, type B (fits on a 384-head, type B)	Molecular Devices	800-635-5577 408-747-1700	9000-0627
Sterile basin for multichannel pipettor (cell seeding)	Fisher	800-766-7000	13-681-101
Non-sterile basin for multichannel pipettor	Fisher	800-766-7000	13-681-100

Calcium Assay Consumables

Calcium Flux Consumables

Item	Suggested Supplier	Phone Number	Item #
FLIPR Calcium Assay Kit	Molecular Devices	800-635-5577 408-747-1700	R8033
FLIPR Calcium 3 Assay Kit	Molecular Devices	800-635-5577 408-747-1700	R8090
Hank's Balanced Salt Solution (10X stock)	Gibco	800-828-6686	14065-056
HEPES buffer solution 1X	Irvine Scientific	800-437-5706	9319
Probenecid, crystalline	Sigma	800-325-3010	P8761
Carbachol (receptor-mediated positive control)	Sigma	800-325-3010	C4382
UTP, Na salt (receptor-mediated positive control)	Sigma	800-325-3010	U6625
Ionomycin (positive control)	CalBiochem	800-854-3417	407950

Membrane Potential Consumables

Item	Suggested Supplier	Phone Number	Item #
FLIPR Membrane Potential Assay Kit	Molecular Devices	800-635-5577 408-747-1700	R8034
540-590 BP FLIPR Filter Kit	Molecular Devices	800-635-5577 408-747-1700	0310-4077
Hank's Balanced Salt Solution (10X stock)	Gibco	800-828-6686	14065-056
HEPES buffer solution 1X	Irvine Scientific	800-437-5706	9319
Carbachol (receptor-mediated positive control)	Sigma	800-325-3010	C4382

Equipment and Supplies Not Supplied with Your FLIPR System

Plate Washer

Plate washer for 96- and/or 384-well plate. We recommend Molecular Devices cell washers.

Other Items Needed or Used

Item	Suggested Supplier	Phone Number	Item #
Multidrop 96/384	Labsystems	800-522-7763 508-541-0444	5840157
12-channel Impact 2 pipettor (15–850 µL)	Matrix	800-345-0206	2014
1250 µL pipet tips for Matrix Impact	Matrix	800-345-0206	8052
Manual12-channel pipettor 50–200 µL	Brinkman		5008130-3
Sterile pipette tips 200 µL	E&K Scientific	408-378-2013	3507-R965
Aspirator manifold, 12-pin	Wheaton Scientific	800-225-1437	851388
Aspirator manifold, 8-pin	Wheaton Scientific	800-225-1437	851381

- 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

Improving Confluence and Adherence of Weakly-Adherent Cells

B

Plate Coating

About Plate Coating

If you are working with a cell line that doesn't grow uniformly on microplates, or that adheres weakly, coating the bottom of the cell plate with a matrix (poly D-lysine, laminin, fibronectin, gelatin, etc.) may improve the homogenicity of the monolayer and may help weakly adherent cells to remain attached to the plate through the washing process. Sample procedures for poly-D-lysine, laminin, and collagen follow.

Poly D-lysine Coating

To coat plates with poly-D-lysine:

Step	Action
1	Prepare a sterile, 100 µg/mL solution of poly D-lysine (Sigma Chemical Co., catalog # P7280) in tissue culture grade water. You will need approximately 8 mL per 384-well plate, or 5 mL per 96-well plate to be coated.
2	Working in a tissue culture hood, sterilely aliquot approximately 20 µL into a 384-well plate, or 50 µL into a 96-well plate, then leave the plate(s) in the hood for 30 minutes.
3	Aspirate the poly D-lysine with a Pasteur pipet, then rinse the plate once with sterile water: 100 µL/well for a 384-well plate, or 200 µL/well for a 96-well plate. Aspirate the water.
4	Allow the plates to dry in the hood before use.

Laminin Coating

To coat plates with laminin:

Step	Action
1	Prepare a sterile, 66.7 µg/mL (500 µg/7.5 mL) solution of laminin (Sigma Chemical Co., catalog # L-6274) in Hank's BSS. You will need approximately 8 mL per 384-well plate, or 5 mL per 96-well plate to be coated.
2	Working in a tissue culture hood, sterilely aliquot approximately 20 µL/well for a 384-well plate, or 50 µL/well for a 96-well plate, then leave the plate(s) in the hood for 30 minutes.
3	Aspirate the laminin with a Pasteur pipet.
4	Allow the plates to dry in the hood before use.

Collagen Coating

To coat plates with collagen:

Step	Action
1	Prepare a sterile, 0.3 mg/mL solution of collagen (Vitrogen 100, from Collagen Biomedical, Palo Alto, CA) in sterile 0.01 N HCl. You will need approximately 8 mL per 384-well plate, or 5 mL per 96-well plate to be coated.
2	Working in a tissue culture hood, sterilely aliquot approximately 20 µL/well for a 384-well plate, or 50 µL for a 96-well plate, then leave the plate(s) in the hood overnight.
3	Working in a tissue culture hood, sterilely aliquot approximately 20 µL/well for a 384-well plate, or 50 µL for a 96-well plate, then leave the plate(s) in the hood overnight.
4	Aspirate the collagen with a Pasteur pipet.
5	The next day, rinse once with sterile PBS 1X to neutralize the pH.

FLIPR Systems Data Processing Algorithms

C

Introduction

In This Appendix

This Appendix describes the algorithms used to process the data on the FLIPR® system. The following topics are covered in this appendix:

FLIPR System Data Processing Algorithms

Overview

A hypothetical experiment is used as an example to describe the data processing algorithms.

These algorithms process the data in order to calculate the following:

- Spatial uniformity correction
- Negative control correction
- Positive control scaling
- Bias value

The manner in which these calculations are derived is explained using the hypothetical example.

Hypothetical Experiment

Experimental Groups

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 (Exper). 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 compound plate to the cell plate and is automatically corrected for by the FLIPR software.)

The data presented is in the same format as the export data files generated using FLIPR. (In this example, only nine wells are considered.)

In the table below, the results of the experiment are presented without **Spatial Uniformity Correction**, **Negative Control Correction**, **Positive Control Scaling**, or **Subtract Bias Value**.

		Well								
Sample	Time	A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exper	A5 Exper	A6 Exper	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
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 fluorescent 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 laser illumination, buffer variations or any other effect which is constant throughout the experimental run. These variations can complicate data interpretation.

Spatial Uniformity Correction Algorithm

About the 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.

Determining Spatial Uniformity Correction

The correction factor is derived by calculating the mean fluorescent counts of all wells at Sample 1 (see below).

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exper	A5 Exper	A6 Exper	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	0.97	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 examples.

Each well-specific spatial uniformity correction factor is calculated by dividing the mean fluorescent counts of all wells by the fluorescent 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, etc.

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).

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exper	A5 Exper	A6 Exper	A7 +Ctrl	A8 +Ctrl	A9 +Ctrl
1	0	8960	8925	8930	8938	8924	8976	8900	8930	8961
2	5	9194	9030	8836	9047	8730	9180	8455	9118	9270
3	10	9408	8925	8648	9265	8730	9282	8633	9118	9682
4	20	9632	8820	8648	49050	48500	42840	50730	48880	55620
5	25	9632	9240	8648	40330	40740	35700	47170	47000	51500

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exper	A5 Exper	A6 Exper	A7 +Ctrl	A8 +Ctrl	A9 +Ctrl
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 fluorescent counts will be skewed by the correction factor. However, the EC_{50} of the agonists tested will not be affected.

Negative Control Correction

About 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 laser power, dye photobleaching, and temperature drifts.

Determining Negative Control Correction

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			Mean	Correction Factor
		A1 – Ctrl	A2 – Ctrl	A3 – Ctrl		
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	A3 – Ctrl	A4 Exper	A5 Exper	A6 Exper	A7 +Ctrl	A8 +Ctrl	A9 +Ctrl
1	0	8960	8925	8930	8938	8924	8976	8900	8930	8961
2	5	9194	8940	8748	8957	8643	9088	8370	9027	9177
3	10	9314	8836	8562	9172	8643	9189	8547	9027	9585
4	20	9536	8732	8562	48560	48015	42412	50223	48391	55064
5	25	9343	8963	8389	39120	39518	34629	45755	45590	49955
6	30	9560	8657	8662	31719	32932	28693	43165	46502	49955

For example, A1 Sample 1 is multiplied by 1.00.

Note The negative control correction factor wells contain the same cells and dyes as the experimental and positive control wells.

Positive Control Scaling

About 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 (i.e., different plates) by controlling factors such as cell density, cell response, laser power, or exposure time. This algorithm also makes EC_{50} comparisons easier.

Determining the Positive Control Scaling

a. The means of all samples of the positive control wells are calculated.

Sample	Time	Well			Mean	Difference From Sample 1
		A7 +Ctrl	A8 +Ctrl	A9 +Ctrl		
1	0	8900	8930	8961	8930	0
2	5	8370	9027	9177	8858	-72
3	10	8547	9027	9585	9053	123
4	20	50223	48391	55064	51226	42296
5	25	45755	45590	49955	47100	38170
6	30	43165	46502	49955	46541	37610

b. The difference in fluorescence counts between Sample 1 and all of the samples is calculated.

c. The greatest difference is determined.

d. 100 is divided by the greatest difference in fluorescence counts (determined above) to give the positive control correction factor i.e. 100/42296 = 0.0024.

e. All samples are multiplied by the correction factor 0.0024 (see the following table)

Sample	Time	Well								
		A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exper	A5 Exper	A6 Exper	A7 +Ctrl	A8 +Ctrl	A9 +Ctrl
1	0	21	21	21	21	21	21	21	21	21
2	5	21	21	21	21	20	21	20	21	23
3	10	22	21	20	22	20	22	20	21	23
4	20	23	21	20	115	113	100	119	114	130
5	25	22	21	20	92	93	82	108	108	118
6	30	23	20	20	75	78	68	102	110	118

Note that 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.

Subtract Bias

About Subtract Bias

The subtract bias algorithm determines the change in fluorescence over background fluorescence. The sample number chosen as background is determined by the user. (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 may dilute the fluorescent 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.

Determining Subtract Bias

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 (see below).

		Well								
Sample	Time	A1 – Ctrl	A2 – Ctrl	A3 – Ctrl	A4 Exper	A5 Exper	A6 Exper	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.

All four algorithms can be used in any combination and activated in any order during analysis or for data export.

Calibrating the Plate Washer

D

Introduction

In This Appendix

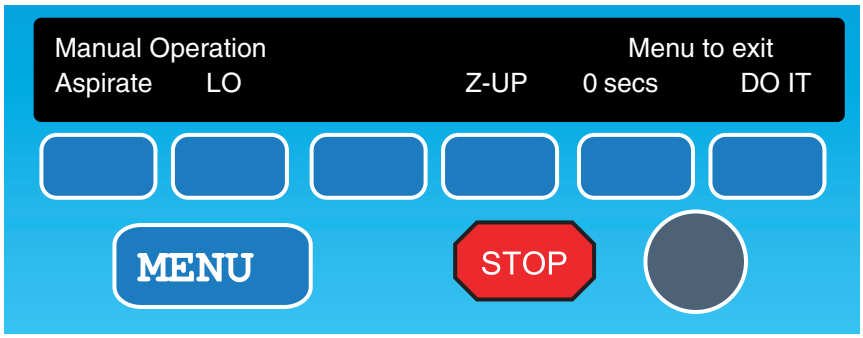
This appendix provides the information and procedures for calibrating the plate washer.

Calibrating the Plate Washer

Procedure for Calibration

Note The Fx factor is set in the factory and does not need to be routinely reset by the user.

To calibrate the plate washer:

Step	Action
1	Select **MANUAL** in the main screen to advance to the **Manual Operation** screen.
2	Select a plate type and put an empty plate in the plate carrier.
3	Select the **NEXT** softkey to advance to the second **Manual Operation** screen. 
4	Select the **ASPIRATE** softkey and continue to press it until **DISPENSE** appears.
5	Choose a dispense volume that is a typical dispense volume for a wash program (e.g. 250 µL).
6	Select the **LO** softkey. Choose the dispense height (LO/HI). It may be necessary to create an appropriate plate definition first. See section *"Setting Up Plate Definitions"* on page 7-18 for instructions on defining plate types.
7	Select the **DO IT** softkey. After completion, select the **Z-UP** softkey and remove the microplate from the plate carrier. Weigh the microplate in grams and calculate the volume of an individual well: $\left(\frac{\text{weight (g)}}{\text{\# of wells}}\right) \times 1000 = \text{volume/well (mL)}$ If the calculated volume is greater than the dispensed volume, decrease the value of Fx in the **CALIB** menu screen and repeat the process. If the calculated volume is less than the dispensed volume, increase the value of Fx in the **CALIB** menu screen and complete the process.
8	When the calculated volume approximates the dispensed volume, the calibration of Fx is complete.

Optical Calibration Protocol

E

Introduction

In This Appendix

This appendix provides the procedure for performing optical calibration of the FLIPR system.

Performing Optical Calibration

When to Perform an Optical Calibration

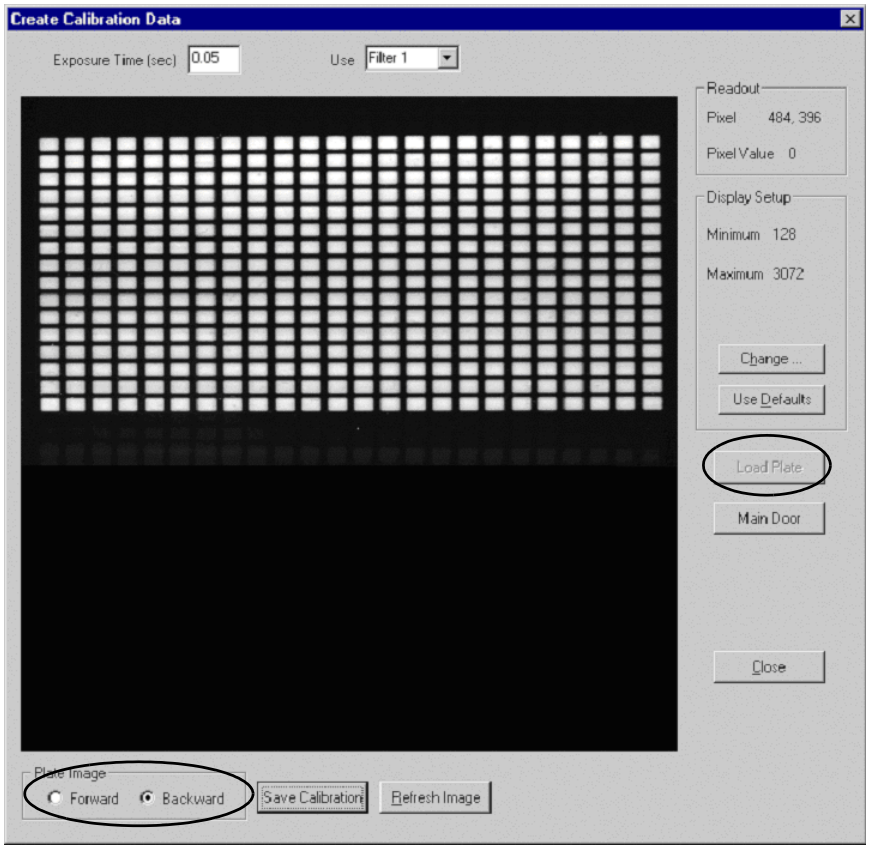
When a calibration data file is applied to a plate read, it will correct for variations in the illumination intensity across the plate. These differences can change under different experimental conditions, and therefore new calibration data may be required.

The frequency at which you should perform an optical calibration depends on how the FLIPR system is used. Typically, we recommend that an optical calibration be performed whenever you have the following situations:

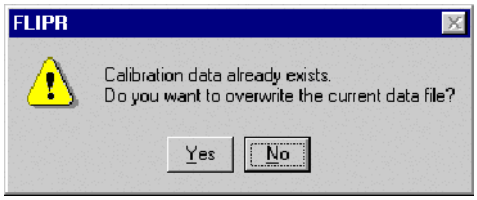
- Major optical maintenance has been performed on the system (in this case the Service Representative will do the calibration).
- Significant changes to the experimental setup has taken place *e.g.*, a large change in the laser power, use of a very different plate type.

Performing the Calibration

To perform an optical calibration:

Step	Action
1	In the FLIPR software main window, select **Calibration** from the **Setup** menu. This opens the **Create Calibration Data** dialog box. **Note** If you are running a calibration for a special experimental setup, then run the calibration under the same conditions *i.e.*, select the **Exposure Time** and **Use** settings that you would use in the experiment. Otherwise, the default values should be adequate.
2	If using a stacker, click **Open Door** or **Load Plate** (if using the stacker). 
3	Load the signal test or calibration plate into the read position (#1). If using the stacker, follow the instructions for loading the plate.
4	Click **Refresh Image**.
5	Click **Main Door** or **Unload Plate** (if using the stacker). Turn the plate 180°**.**
6	Select **Forward** or **Backward** in the **Plate Image** panel to reflect the change in plate orientation.
7	Click **Refresh Image**.

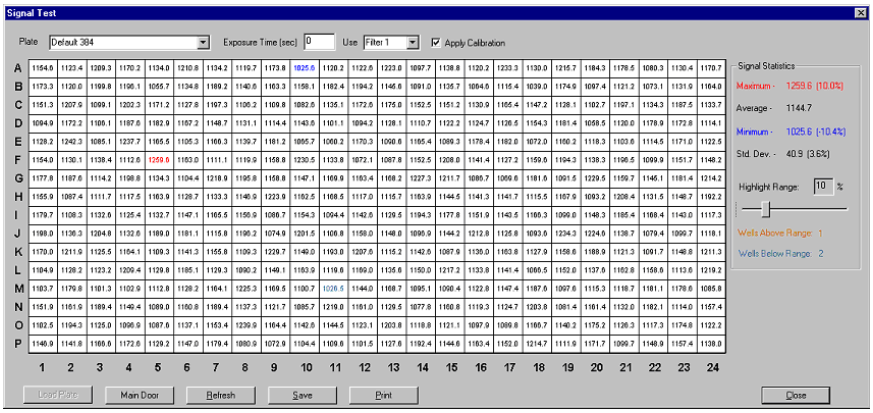
To perform an optical calibration: *(continued)*

Step	Action
8	Click **Save Calibration**. If you already have a calibration file, you will see the following message. To overwrite the existing calibration, click **Yes**. 

Checking the Calibration

After you create a new optical calibration data file, you may check how it works using the signal test command.

To check the calibration using the signal test:

Step	Action
1	With your plate still loaded in the FLIPR system, select **Run Signal Test** from the **Run** menu. 
2	Select the appropriate plate from the **Plate** drop-down menu and enter the **exposure time** that you used for the calibration collection.
3	Click **Refresh**.
4	To apply the newly collected calibration data file, check **Apply Calibration**. Examine the values in the **Signal Statistics** panel. The **Std Dev** should be at or less than 3.5%.
5	If the value is greater than 3.5%, check for visual anomalies in the **Plate Viewer** under the **Run** menu.
6	Check the plate alignment as defined in the **Plate Library** under the **Setup** menu.
7	Repeat signal test as defined in steps 1 and 2 with **Apply Calibration** unchecked. Examine the values in the **Signal Statistics** panel. The **Std Dev** should be at or less that 8.0%.

To check the calibration using the signal test: *(continued)*

Step	Action
8	If the value from step 7 is less than 8.0%, repeat the calibration as defined in *"Performing the Calibration"* on page E-3. Then repeat checking the calibration as described in steps 1 to 4.
9	If after repeating the calibration, the standard deviation is still not within specifications, call Molecular Devices Technical Support.

Decontamination Certificate

F

Decontamination Certificate

Procedure for Completing the Certificate

Circle the appropriate statement and complete both pages of the form as necessary.

a. The instrument or parts that are being returned for rework were not used for any application involving blood or other potentially infectious material.

b. The instrument or parts that are being returned for rework have been decontaminated.

c. The instrument or parts that are being returned for rework have NOT been decontaminated. Every effort must be made to decontaminate before returning instruments or parts to the factory. Include a statement that identifies the type of potentially infectious materials involved, e.g. blood or other body fluids, as well as why no decontamination was performed:

_____

_____

_____

_____

_____

This is to certify that this part or instrument has been prepared for shipment by me or someone under my supervision.

Signature: _____

Name (Print)_____

Date _____

Part # _____ Model # _____ Serial # _____

Nuclides Used: _____

Biologicals Used (i.e. human serum, body fluids): +_____

_____

Customer Contact Name: _____

Institution: _____

Phone # _____ Fax # _____

Enclose this sheet with the part or instrument being shipped to another location or returned to the factory. Please enclose the sheet in such a manner that it can be easily read when the package is opened.

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