FLIPR384

Fluorometric Imaging Plate Reader

User’s Manual

Molecular Devices

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Chapter 1
System Overview

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1.1 Introduction

The FLIPR^{384} and FLIPR^{96} 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 being used in many types of assays, such as those designed to monitor changes in membrane potential, in intracellular Ca^{++} flux, and intracellular pH.

1.2 System Description

The FLIPR system's basic principle of operation is to illuminate the bottom of a 384- or 96-well plate and simultaneously 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 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."*

1.3 System Components

1.3.1 The FLIPR^{384} 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.

1.3.2 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 cell plate (either a 384- or 96-well plate).

The simple stage allows you to run the system manually or integrated to a linear track 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 integrating 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 precise 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.

1.3.3 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.*

1.3.4 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.

1.3.5 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.

1.3.6 The Plate Stacker and Washer

The stacker and washer are stationed on a supporting 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.

1.3.7 The Host Computer

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

1.4 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.

1.4.1 Electrical

A single 110 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. In Europe, a transformer is supplied to convert the room outlet to 110 VAC. 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.

1.4.2 Physical

a) 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.

b) 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.*

c) Air

The FLIPR system requires an air source that can produce a steady supply of clean air at 85 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.

Chapter 2
System Hardware Features

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2.1 System Diagram

This chapter provides brief descriptions of the hardware features available with the FLIPR system. These features are shown in Figure 2-1.

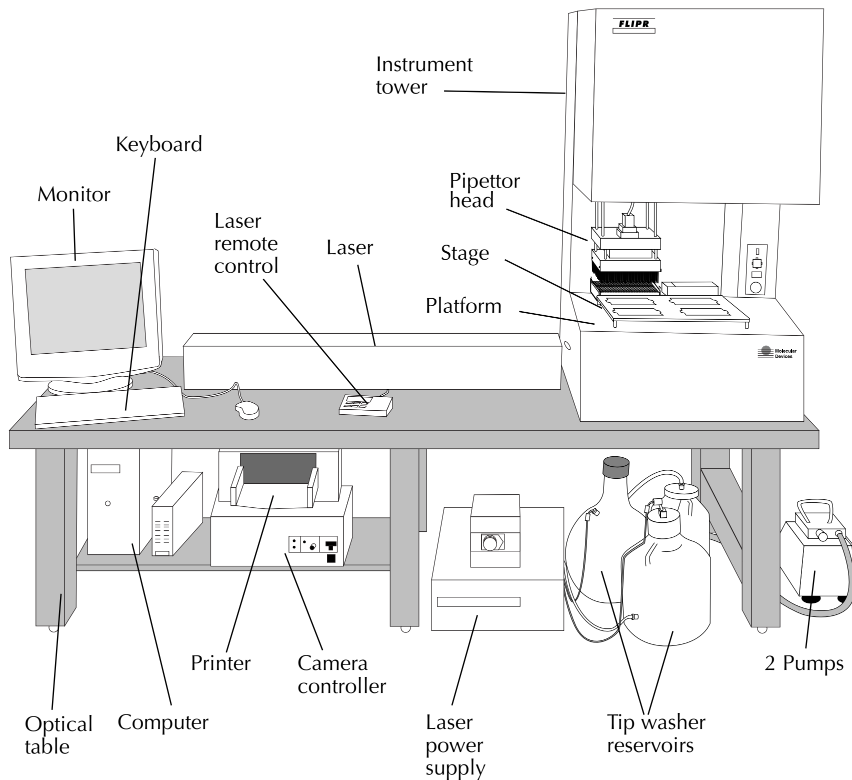

Figure 2-1: The FLIPR system.

2.2 Six-position Platform

The 96- and 384-well plates are placed in the FLIPR system's 6-position platform (Figure 2-2) according to the following pattern:

- 1 cell plate 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)

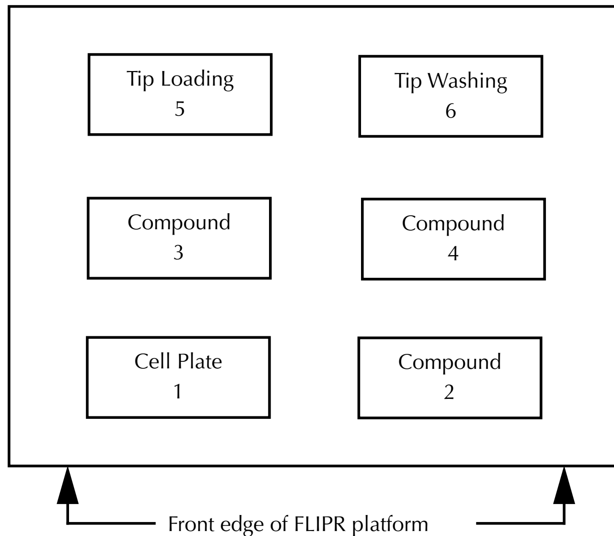

Figure 2-2: The FLIPR six-position platform.

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 feet have locating pins that protrude and fit into corresponding holes in the platform.

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

2.2.1 Simple Stage

This stage is used for manual and robot-integrated operation of the FLIPR system and has four defined positions for micro-

plates (see Figure 2.2.1). All plates and tips are positioned by the user or the robot. One position (Position 1) is assigned to the cell plate, and the remaining three positions are assigned to compound plates. Up to three compound plates can be used in a single assay.

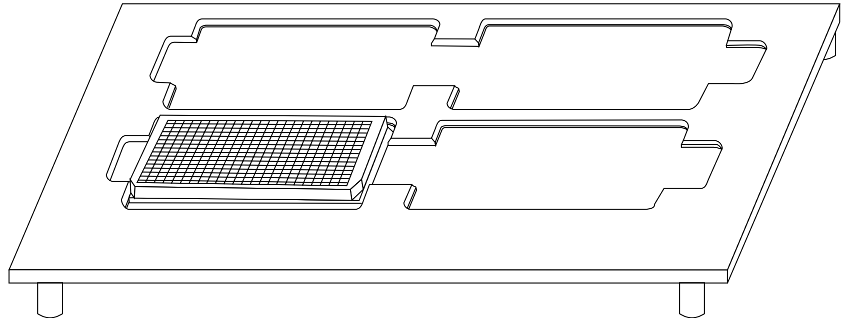

Figure 2-3: The simple stage.

If a 384-well cell plate is used, Position 1 is open and the 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."*

2.2.2 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 (Figure 2-4).

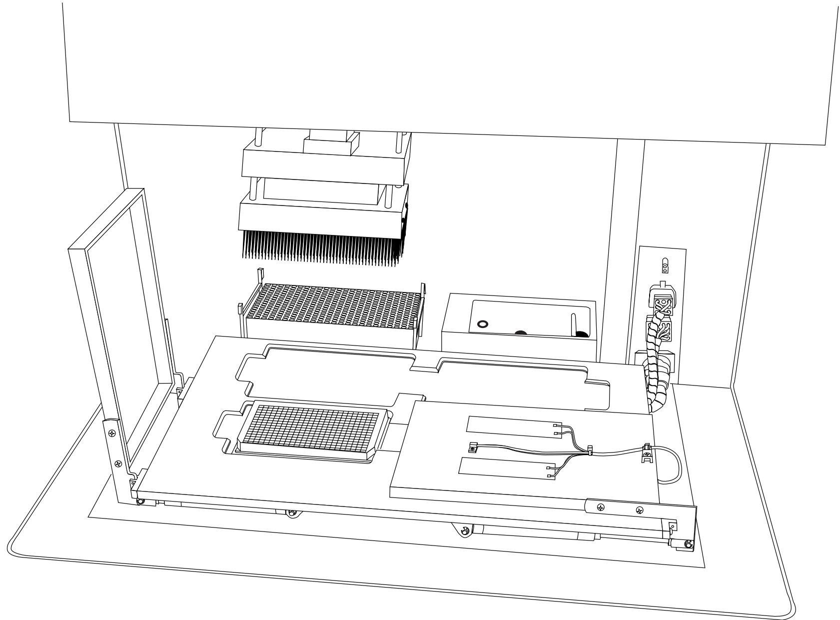

Figure 2-4: The heated stage.

In Positions 1 and 2, the temperature is precisely 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.

Note: *FLIPR^{384} and FLIPR^{96} 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 (+5º C) to 40º 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:

1) Clamshell lids close over Positions 1 and 2.
2) Pipettor picks up the pipette tips from Position 5 and moves to Position 6.
3) Clamshell lid opens over Position 2.
4) Pipettor moves to the compound plate in Position 2.
5) Pipettor picks up fluid and repositions itself over Position 3.
6) Experiment starts and the initial fluorescence is measured.
7) When programmed for fluid addition, the lid over Position 1 opens, the pipettor repositions itself over the cell plate and delivers fluid.
8) 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.*

2.2.3 Stacker Stage

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

2.3 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). 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.

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.*

2.3.1 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 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.

Cautions: *Make sure that the tip rack is seated against the back of the holder.*

Molecular Devices strongly recommends that you only use 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.

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

NEVER, under any circumstance, touch the tip rack in the holder once the tips are loaded. If the tip rack is dislodged between the beginning and the end of the assay, the pipettor will be unable to return the tips to the rack.

2.3.2 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 white teflon wash basin. Tip washing can be programmed to occur at the end of an experiment or after the last fluid addition.

The wash basin is connected to the wash solution supply, a waste reservoir and an overflow reservoir. The containers are usually located behind 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 basin is shown in Figure 2-5.

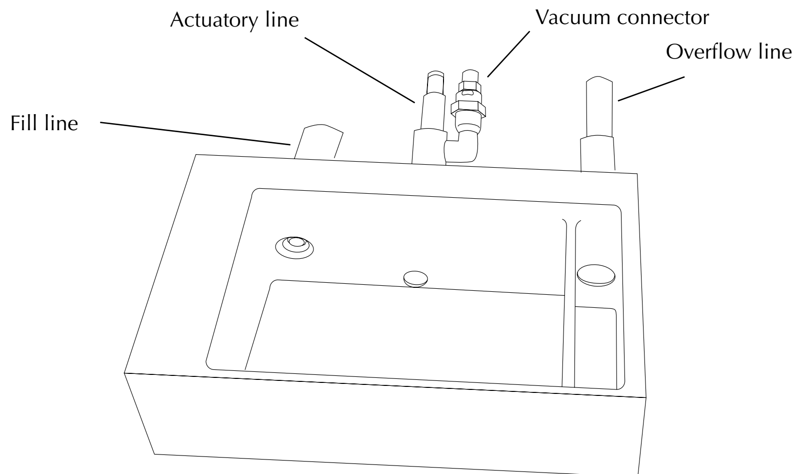

Figure 2-5: The wash basin.

The following wash parameters can be controlled through the software:

- Wash volume in tips
- Wait time of wash solution in tips
- Speed of wash fluid ejection
- Number of pipetting cycles
- Basin volume rinse
- Number of wash cycles (the number of times the wash basin refills and the tip-wash cycle restarts)

Each new assay may require that the wash solution be modified and the wash cycle parameters be optimized. The exact composition of the wash solution may change depending on the compound being used. A mixture of deionized water with 10% ethanol or DMSO has been proven successful.

2.4 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.

2.5 Emission Filters

A 2.0-inch 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.

2.6 CCD Camera

The CCD camera is located beneath the cell and compound plate platform. 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 -30 °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, in turn, 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 2. A small door, located at 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.*

2.7 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.

Warning: *Never remove the laser enclosure while the laser is in operation or try to observe the laser radiation beam directly.*

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 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. You can modulate both the amperage and wattage by pressing the UP/DOWN arrows on the remote control (see Figure 2-6).

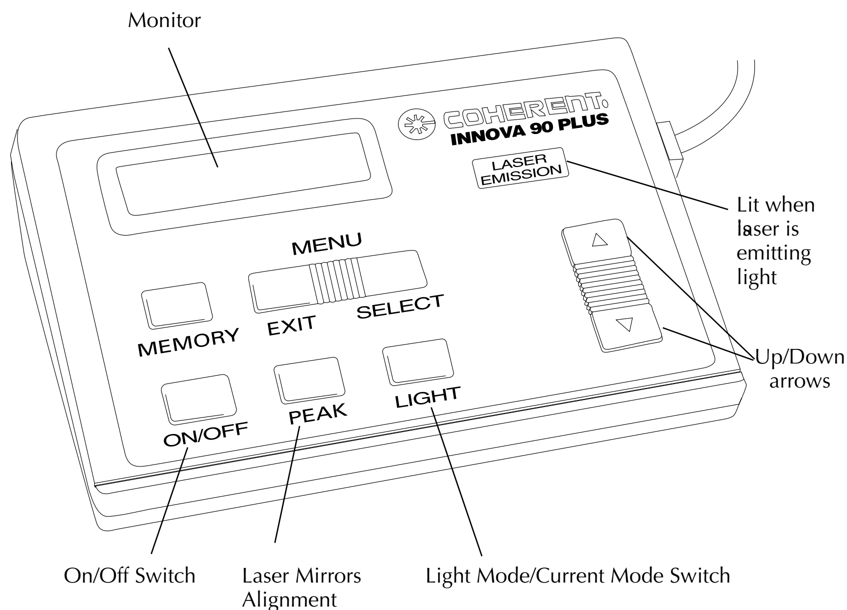

Figure 2-6: Laser remote control.

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.

2.8 Plate Stacker and Washer

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

2.9 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
- 64 MB of RAM (or above)
- Hard Disk Drive (4 GB or above)
- SCSI controller
- Ethernet interface
- 2 GB Jaz Drive
- 17-inch monitor
- Inkjet printer

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.

Chapter 3

System Start-up and Shut-down Procedures

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3.1 Start-up Procedure

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.

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 Figure 3-1). 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.

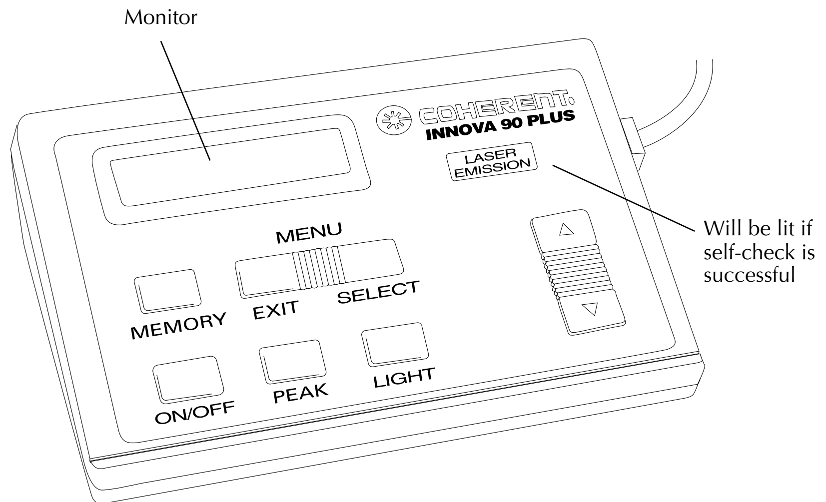

Figure 3-1: Laser remote control — the emission light will be lit if the laser passes its self-check.

The laser typically requires approximately 30 minutes 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 the FLIPR system. The power switch is located at the rear of the tower.

7) 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).

8) Turn on the vacuum and peristaltic pumps.

9) Turn on (or open) the air source and set the air pressure to at least 85 PSI.

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.*

3.2 Shut-down Procedure

1) At the end of a programmed experiment or after stopping an experiment from the **Stop** pull-down menu, wait for any pipettor operation to complete and the door to open.

2) Exit the FLIPR software by choosing **Exit** from the **File** menu.

3) 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.*

4) Turn off (or close) the air source.

5) Turn off the vacuum and peristaltic pumps.

6) Turn off the FLIPR system power switch.

7) Turn off the camera controller, the computer and monitor.

8) After a minimum of 5 minutes, turn off the laser's cooling system.

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4.1 Introduction

This chapter provides a description of the program windows, menu commands and dialog boxes in the FLIPR^{384} control software and gives an overview of their functions. It also details how to use the software to set up and control the hardware.

4.2 Software Start-up

Before launching the FLIPR control software, check that the hardware components are powered on according to the procedures described in Chapter 3, *"System Start-up and Shut-down Procedures."* When the software is started, the program window shown in Figure 4-1 will be displayed on the computer monitor.

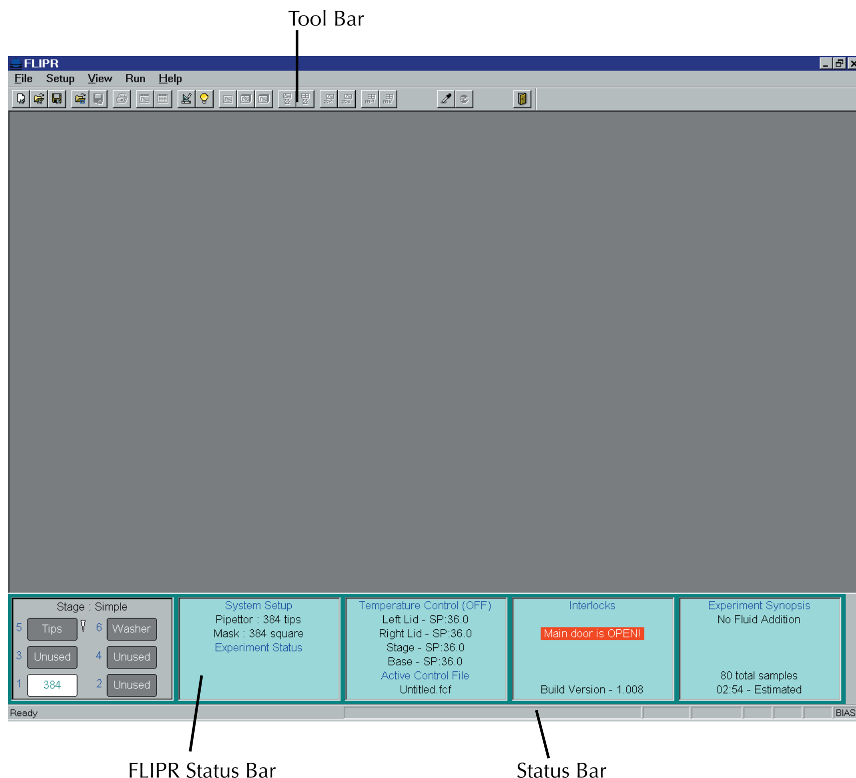


Figure 4-1: The initial FLIPR^{384} program window.

At the bottom of the window is the FLIPR Status Bar, which displays information about the instrument and the experiment protocol. This includes:

- Type of stage used and the activated plate positions
- Type of head (96- or 384-well) and plates (96-well, 384-well or boat)
- Temperature control status (heated stage) and active template file used (control file or .fcf file)
- Status of interlocks
- Experiment synopsis, including the number of fluid additions and the estimated experiment run time

Below the FLIPR Status Bar is a status bar that displays the name of the active group of data plots and any graph processing functions being used. If you are using the cursor to step through the time points on the graph, the Status Bar will display the position of the cursor (based on the sample number), the time, the bias value (see *"Subtract Bias Based on Sample"* on page 67) and the delta (fluorescence counts).

At the top of the window, the Menu Bar and the Tool Bar (shown in more detail in Figure 4-2) are displayed. The Tool Bar symbols are shortcuts for functions accessible through the Menu Bar. All FLIPR^{384} System operations are controlled by making selections from either the Menu Bar or the Tool Bar.

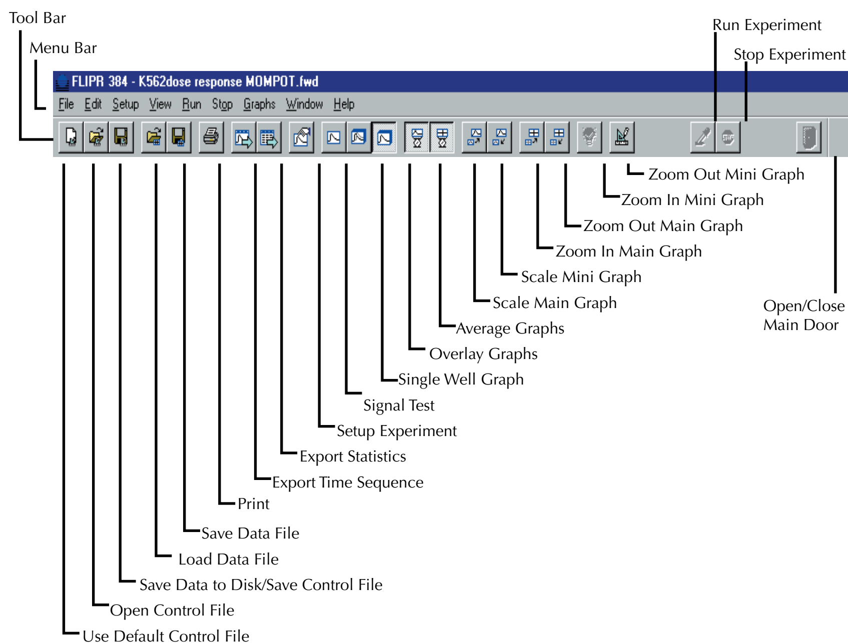


Figure 4-2: The FLIPR^{384} Menu Bar and Tool Bar.

4.2.1 Defining the Hardware Setup

Before running an experiment, the components of the FLIPR^{384} System must be defined in the software. If the physical setup of the instrument is the same as the previous assay, you don't have to re-define the hardware setup. A change in stage or tip washer requires that you define the hardware setup. Choose **Hardware** from the **Setup** menu to open the **Hardware Setup** dialog box. This allows you to specify the components being used (Figure 4-3).

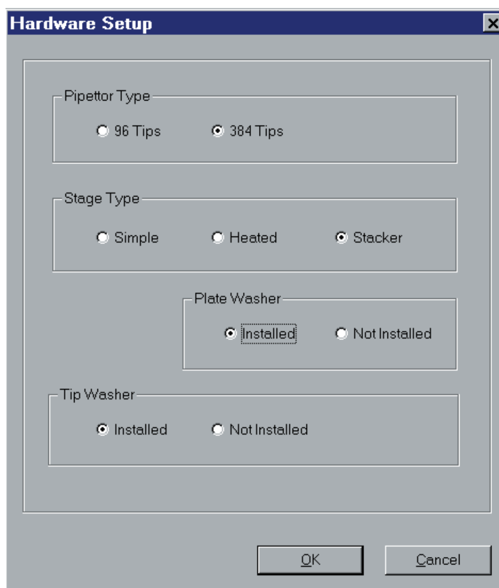


Figure 4-3: The **Hardware Setup** dialog box.

Please refer to Section 4.4.6, "*Hardware Setup*," starting on page 55, for a complete description of the functions in the **Hardware Setup** dialog and how to use them.

4.3 The FLIPR^{384} Control Software Menus

The following sections explain each menu item and dialog box in the FLIPR^{384} Control software. Some dialog boxes can contain different options depending on the stage/pipettor head used. In these cases, each option will be detailed.

Note: *All of the FLIPR^{384} Control software options can be saved in a unique* **.fcf** *template file.*

The order in which the menu items are discussed is not the order in which they appear, from left to right, on the Menu Bar. Rather, it is the order in which you would typically choose them while setting up an experiment. The **Setup** menu allows you to define all the experimental parameters. The **Run** and **Stop** menus start and stop the experimental run, respectively. The **Graph**, **Window** and **View** menus control the

display of the data. The **File** and **Edit** menus control the export and print functions, as well as management of the template files.

4.4 The Setup Menu

The **Setup** menu is shown in Figure 4-4.

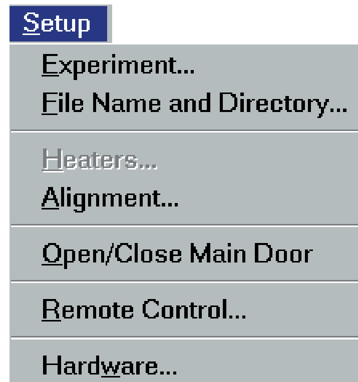


Figure 4-4: The **Setup** menu.

4.4.1 Experiment

Selecting this menu item will bring up the **Experiment Setup** dialog box. This contains eight tabs of options used to define the experimental setup (see Figure 4-5). Be sure to set all of the options you want to use in each tab. Clicking the **OK** button will set all of the choices you have made and close the dialog box.

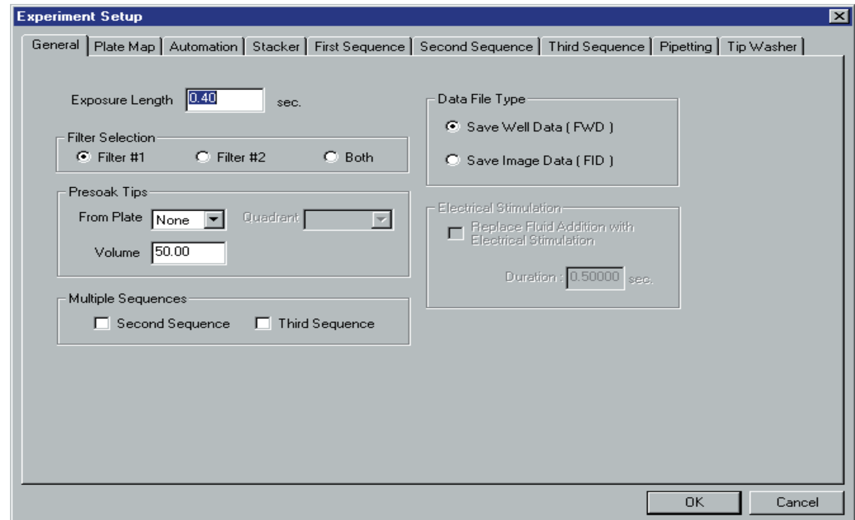


Figure 4-5: The **Experiment Setup** dialog box.

a) General

The **General** tab contains options that allow you to define general experiment parameters (refer to Figure 4-5.)

- **Exposure Length**
  This field displays the time that the camera's shutter aperture is open (in seconds). The exposure time will influence the fluorescence signal recorded. Most fluorescence assays for calcium are run using an exposure time of 0.4 seconds. If the initial signal obtained is higher than desired, the exposure time can be decreased. The minimum exposure time that can be entered is 0.05 seconds. If you need to increase the initial signal (i.e., to collect more light), you can increase the exposure time. The maximum exposure time is 5 seconds. The exposure length also determines the update time for data collection. The camera requires 0.6 seconds to integrate the data from each exposure. The highest update frequency for an exposure length of 0.4 seconds is once per second.

- **Filter Selection**
  This panel selects the filter in the "Filter #1" slot by default. Most of the standard fluorescence dyes used with the FLIPR[384] System will work with this filter. This filter has a bandpass of 510 to 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.

- **Presoak Tips**
  This panel allows you to specify the duration and volume of a presoak prior to the first addition. Presoaking is generally used in membrane potential assays, using the dye DiBAC, which adheres to plastic. The default duration for presoaking is 1 minute, but the experiment can be started sooner by clicking the **Run** button.

Note: *Do not use the same 384-well plate for presoaking and for compound addition. If using 96-well plates, the same plate can be used for presoaking and for compound addition.*

- **Multiple Sequences**
  This check box allows you to activate a second and third fluid addition. Note that these additions must also be activated from the **Second Sequence** and **Third Sequence** panels, respectively.

- **Data File Type**
  This panel allows you to choose between two file formats. The FWD format (FLIPR Well Data) file contains the fluorescence intensities saved as fluorescence count numbers and the graphic plots of the experimental data. The file is typically 30–50 kilobytes in size for a 4–5 minute assay. The FID format (FLIPR Image Data) contains the graphic plots *and* all of the well images taken during the experiment. This allows you to visually replay the experiment later. A FID file is typically several megabytes

in size for a 4–5 minute assay. The default setting is the FWD format which creates smaller, easier to store files. FID files are preferred during assay development to check for dye leakage and whether the cells are being dislodged from the wells.

b) Plate Map

The **Plate Map** tab displays a set of options that define the number and type of compound plates used in the assay (see wells or reservoir "boats", Figure 4-6).

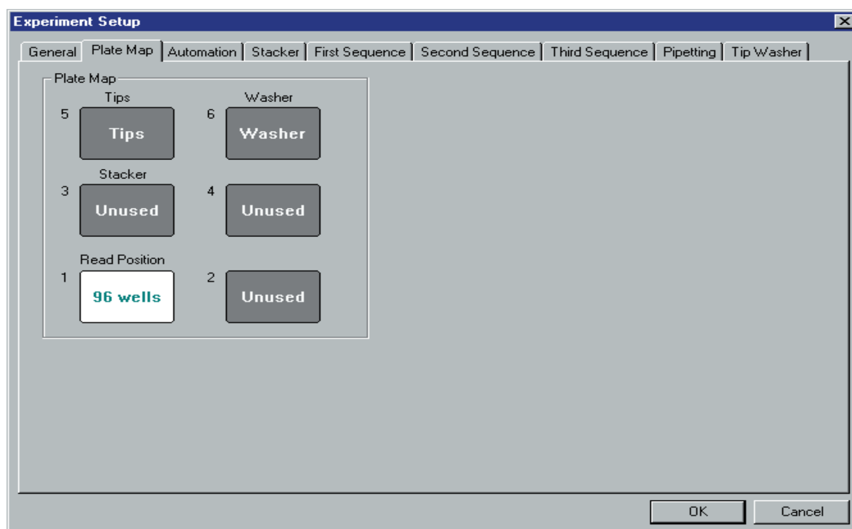


Figure 4-6: The **Plate Map** tab of the **Experiment Setup** dialog box.

Note: *If a 96-well pipettor head is used, the cell plate can be either 384- or 96-well.*

The **Boat** *option can be used for 96-well plates if the system is using a 96-well pipettor head, or for 384-well plates if the system is using a 384-well head.*

To define the plate type, simply click on the desired plate position to toggle between the available formats. Each click will cause the next available plate format to be displayed in the plate position.

c) Automation

The **Automation** tab allows you to automate some functions such as printing, data export and tip washing (Figure 4-7).

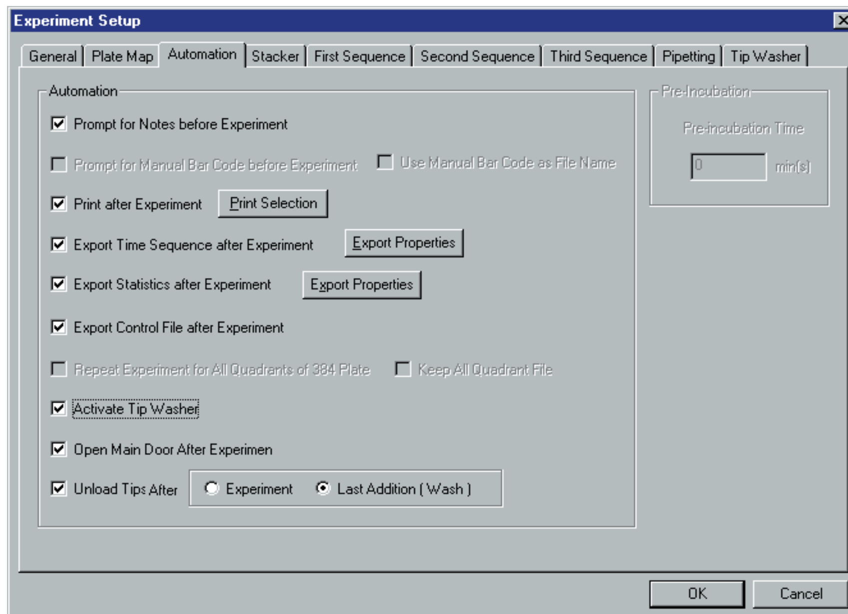


Figure 4-7: The **Automation** tab of the **Experiment Setup** dialog box.

Automation

This panel lists the options available for any stage used. Options that are not available will be grayed out.

- **Prompt for Notes before Experiment**
  Checking this box triggers the **Notes** dialog box to appear immediately after starting an experiment. You can then type in the **Notes** box and click the **OK** button to save what you've typed, or you can cancel it. The "notes" are a text file attached to the data file that can contain relevant information regarding the assay.

- **Prompt for Manual Bar Code before Experiment**
  This option enables or disables an integrated barcode reader function.

- **Use Manual Bar Code as File Name**
  When active, this option automatically uses the bar code as the file name.

- **Print After Experiment**
  If checked, this option will automatically print the reports at the end of an experiment, according to the print selection made prior to the experiment in the **File** menu (see "*File Names*" on page 49.)

- **Export Time Sequence/Statistics/Control File after Experiment**
  These options automatically export the selected data to a folder designated in the **File Names** menu item of the **Setup** menu (see "*File Names*" on page 49.) The export parameters and export destination are determined prior to starting the experiment. For more on exporting data, see "*Export*" on page 81.

- **Repeat Experiment for All "Quadrants" of 384 Plate**
  This option allows you to automatically perform an experiment four times, once in each quadrant of a 384-well cell plate, with a 96-tip pipettor. The compound addition plate can be a 96-well, 384-well or boat plate.

- **Activate Tip Washer**
  This option must be checked for the tips to be washed at the end of the experiment. The specifics of the tip-washing program are defined using the options in the **Tip Washer** tab of the **Experiment Setup** dialog box.

- **Open Main Door After Experiment**
  This option is selected when using the simple stage or the heated stage, since you need access to the cell and compound plates between assays.

- **Unload Tips After**
  If this option is unchecked, the tips remain on the pipettor head at the end of the experiment. If checked, the tips are

unloaded at the end of the experiment or after the last addition as specified by the corresponding buttons.

Note: *The tips can also be unloaded by selecting* **Run→Special Operations→Unload Tips**. *In this case, the tips will be unloaded to the rack or plate position 4.*

d) Stacker

The **Stacker** tab contains options that control the stacker and the plate washer (Figure 4-8).

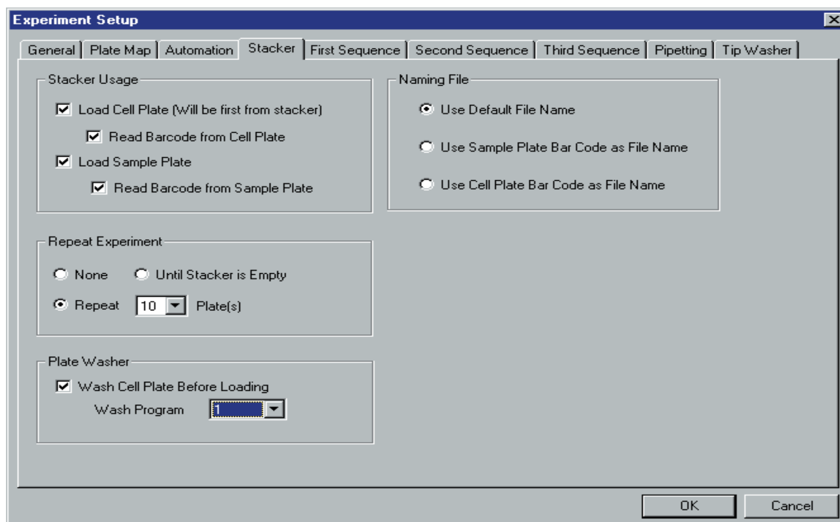


Figure 4-8: The **Stacker** tab of the **Experiment Setup** dialog box.

- **Load Cell Plate**
  This option directs the stacker to load the cell plate into the Read position.
- **Read Barcode from Cell Plate**
  This option directs the stacker to read the barcode on the cell plate.
- **Load Sample Plate**
  This option directs the stacker to load the compound plate into position 3 of the stacker stage.

- **Read Barcode from Sample Plate**
  This option directs the stacker to read the barcode on the sample plate.

- **Naming File**
  This option allows you to choose the format of the file name using either the default name, the sample plate bar code or the cell plate bar code.

Note: *The bar code labels for both plates are saved with the data file, independent of the* **Naming File** *parameter chosen.*

- **Repeat Experiment**
  This option provides automation options for the run. You can choose to stop the FLIPR^{384} System after each run, run a specified number of experiments and then stop, or continue running until the stacker cassette is empty.

- **Plate Washer**
  When activated, this option directs the stacker to shuttle the cell plate into the plate washer prior to moving it to the stacker stage. The wash protocol specified in the **Wash Program** field will be used to wash the plate. Wash protocols are created and stored in the plate washer software (see Chapter 7, *"The Plate Washer,"* for complete details).

e) First Sequence/Second Sequence/Third Sequence

The **First Sequence**, **Second Sequence** and **Third Sequence** tabs are identical. The following panel descriptions apply to all three. The **First Sequence** tab is shown in Figure 4-9. The options in these tabs allow you to define all of the parameters for the compound addition into the cell plate.

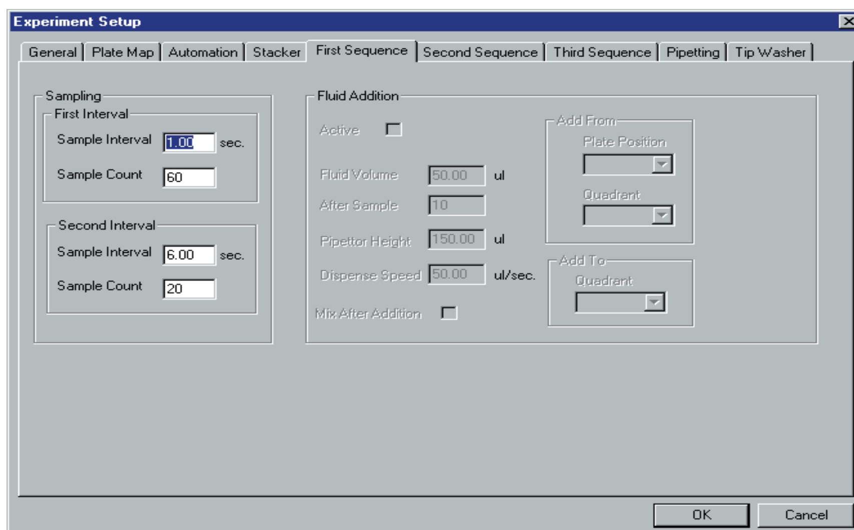


Figure 4-9: The **First Sequence** tab of the **Experiment Setup** dialog box.

The **Second Sequence** and **Third Sequence** tabs will be activated only if the corresponding boxes in the **Multiple Sequences** panel of the **General** tab have been checked. Next, the **Active** boxes in the appropriate sequence tab will need to be checked. Make sure that each sequence of fluid addition and pipettor height has been properly defined using the options detailed below.

Sampling

The **Sampling** panel is located on the left side of the **Sequence** tabs. It contains options that define the update intervals and the duration of the sequence.

- **Sample Interval**
  This field refers to the time between two pictures or "samples" of the fluorescence signal. The minimum sample interval is determined by the exposure length. For more information, see *"General"* on page 36.

- **Sample Count**
  This field refers to the number of pictures taken during each interval. In Figure 4-9, the **First Interval** and **Second Interval** panels indicate that a picture is taken

every second for 1 minute (60 counts), then a picture is taken every 6 seconds for another 2 minutes (20 counts). The reason there are two sampling intervals is because each file is limited to a total of 300 picture frames per file (including all 3 fluid additions). For most assays, it is most important to record the signal at the onset of the cell response and less important during the later stages of the response. For this reason, the first sampling interval can be used for rapid image updates, and the second interval can be used for less frequent updates.

Fluid Addition

The **Fluid Addition** panel is on the right side of the **Sequence** tabs. Check the **Active** box to make the options in this panel available.

- **Fluid Volume**
  This value is dependent on the design of the experiment and will vary from assay to assay. The minimum and maximum working volumes are 1–25 µL for 384-well plates and 5–200 µL for 96-well plates. Please refer to specific application notes for recommendations on the fluid volumes to use.

- **After Sample**
  This field enables you to select the number of basal fluorescent images collected before the fluid addition. Most assays collect 10 basal images before the fluid addition.

- **Pipettor Height**
  This field represents the location of the pipettor tips prior to the fluid addition. Typically, the tips are positioned above the meniscus of the fluid present in the cell plate at the beginning of the assay. When the pipettor takes up the predetermined volume of fluid from the compound plate, it aspirates a small air bubble to prevent any loss of compound during

the pipettor movements. If the tips are placed below the meniscus, dispensing the compounds could cause perturbations in the reading. The introduction of the air bubbles might result in diffraction of the fluorescence signal.

It is also recommended that the pipette tips be immersed in the fluid by the end of the compound addition. This minimizes optical perturbation in the well during the course of the assay. Leaving the tips in the fluid prevents any risk of liquid drops hanging from the tips and dripping into the wells later in the assay. However, the tips may be removed to the tip washer during data collection, if desired.

- **Dispense Speed**
  The values in the **Dispense Speed** field must be optimized for each type of assay. 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 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.

Note: *Selecting an optional dispense speed is discussed in greater detail in Chapter 5, "How to Run an Experiment."*

- **Mix After Addition**
  If mixing after fluid addition is required, check this box. Additional mixing parameters must be defined in the **Pipetting** tab of the **Experiment Setup** dialog box. See *"Pipetting"* on page 46. Mixing is not recommended if rapid updates (<5 seconds) are being recorded.

- **Add From and Add To**
  These panels are not active unless the plate map has been defined. The options available depend on what has been defined in the plate map dialog box. You can enter 2, 3 or 4 into the **Add From Plate Position** field. The **Add From Quadrant** field is available only if the instrument has a 96-tip pipettor, the compound plates are 96-well plates or boats, and the cell plate has been defined as a 384-well plate.

Caution: *Optimal pipettor head alignment is critical for setting these parameters.*

The quadrant design is shown in Figure 4-10.

Figure 4-10: Quadrant design.

The **Add To** panel is available only if the compound plates are 96-well plates or a boat, and the cell plate is a 384-well plate.

f) Pipetting

This tab contains options that control mixing, tip positioning and fluid addition (Figure 4-11).

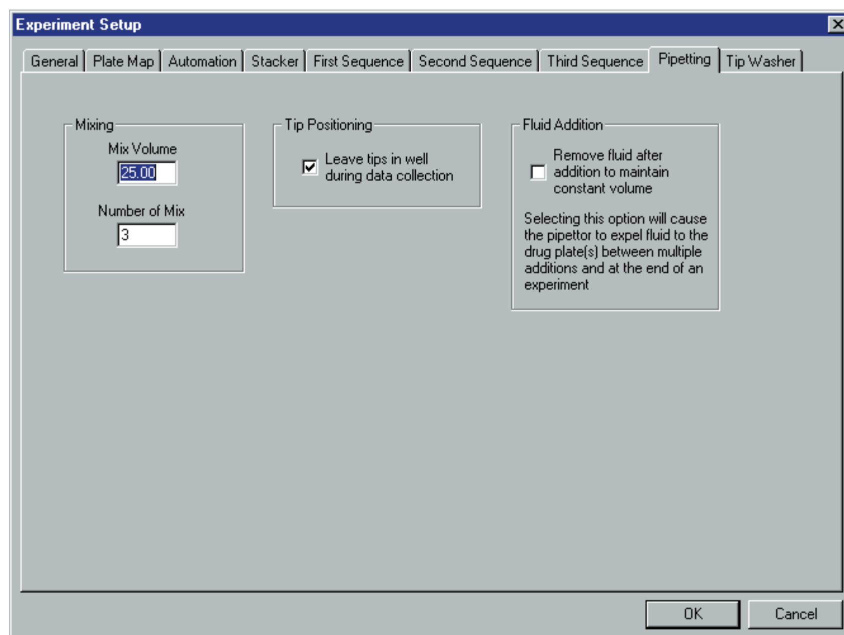


Figure 4-11: The **Pipetting** tab of the **Experiment Setup** dialog box.

- **Mixing**
  The value entered in this field must be empirically determined for each assay. Mixing is done by pipetting up and down a defined number of times. The greater the volume and number of mixing cycles, the more efficient the mixing of compounds in the cell plate. The mixing speed and pipettor position during mixing are by default the same as dispensing fluid to the cell plate.

- **Tip Positioning**
  This field allows you to choose between leaving the tips in the wells after compound addition or moving the tips to the tip washer or "home" position. The default setting is to leave the tips in the wells. Leaving the tips in place may create less perturbation of the compound addition. You can modify this setting during the course of optimizing the assay.

Note: *When using the heated stage, the tips must be removed and the pipettor head must return to its "home" position before the stage's clamshell doors are closed.*

- **Fluid Addition**
  When this option and the mixing option are both checked, the pipettor dispenses the fluid in the cell plate, mixes it, then aspirates the same volume out of the cell plate wells before the experiment proceeds. This option's purpose is to keep the assay volume constant.

g) Tip Washer

The **Tip Washer** tab is shown in Figure 4-12. The options in this tab will not be active until the **Activate Tip Washer** box in the **Automation** tab has been checked.

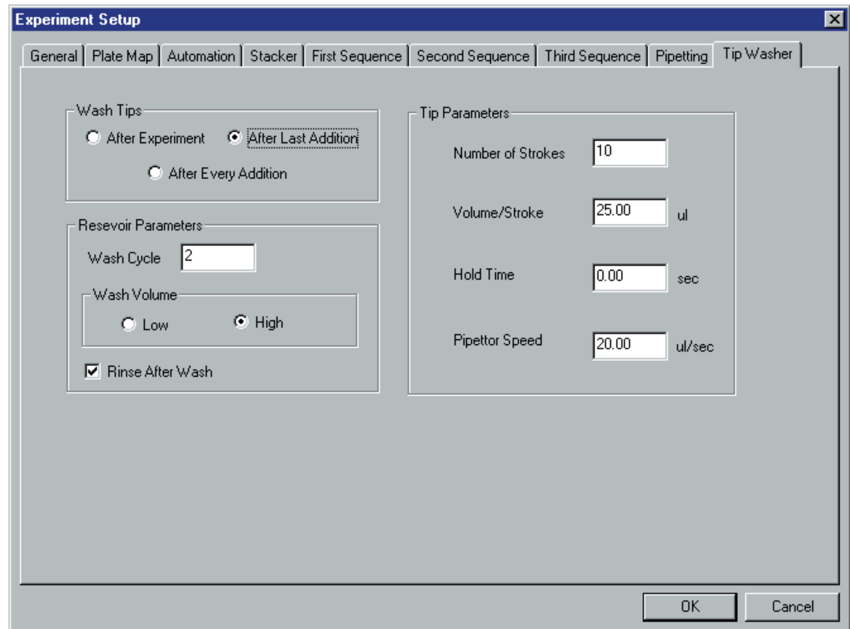


Figure 4-12: The **Tip Washer** tab of the **Experiment Setup** dialog box.

- **Wash Tips**
  In this panel you can choose to program the tip wash at the end of an experiment, after the last fluid addition or after each fluid addition.

- **Tip Parameters**
  The fields in this panel define the pipetting operation. This includes the number of strokes, the pipetted volume, the hold time for each fluid intake, and the pipettor speed of both fluid intake and fluid dispensing in the wash basin.

- **Reservoir Parameters**
  This field defines the number of wash cycles (i.e., the number of times the entire **Tip Parameters** cycle is repeated), the wash fluid volume in the wash basin (low is 2/3 full, full is 2 mm from the top) and whether or not there is a rinse cycle after the wash cycle. The rinse consists of spraying the tips and basin after the end of the last cycle. The reservoir fills up with fresh wash fluid between wash cycles.

Caution: *Make sure the wash fluid reservoir is sufficiently full before running an assay and tip washing. Warning messages will appear if the fluid supply is low or if the waste reservoir is close to full.*

4.4.2 File Names

The FLIPR^{384} System control software **automatically** assigns a name to each data file and saves it at the end of an experiment. The file-save function cannot be disabled. The **File Names** dialog box allows you to define the type of names generated and the destination of the files saved (see Figure 4-13).

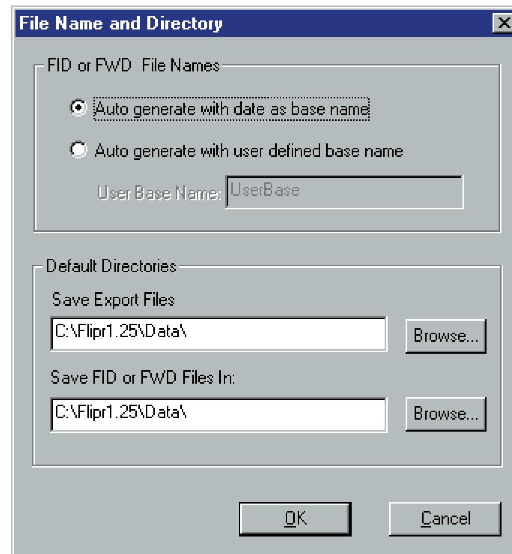


Figure 4-13: The **File Names** dialog box.

- **FID or FWD File Names**
  Two options are available in this panel. You may auto-generate the name based on the date (MMDDYYYY_nx) or write another base name for the files (abcdef_nx). The file-naming system uses the chosen base name and a number increment (x) attached to it. Most users choose to name the files after the date, since it makes the data management a lot easier than alternative systems. The increment number is automatically assigned, and starts at 1.

- **Default Directories**
  Enter the destination paths for saved and exported files in this panel. We recommend you save all FID and FWD files in a common directory to ensure all experiments are present for archiving. Users can send the data export files to personal directories for more efficient retrieval.

Caution: *Before entering the file destination, check that the directory you are specifying exists.*

If the auto-export function is selected in the **General** tab of the **Experiment Setup** dialog box, the exported file is automatically stored in the folder defined in the **Export Files** field of the **File Names** dialog box.

4.4.3 Heaters

The **Heaters** menu item is available only when the heated stage is used. It opens the **Setup Heaters** dialog box shown in Figure 4-14.

Caution: *Contact Molecular Devices before using any of the options in the* **Setup Heaters** *dialog box to change the temperature parameters.*

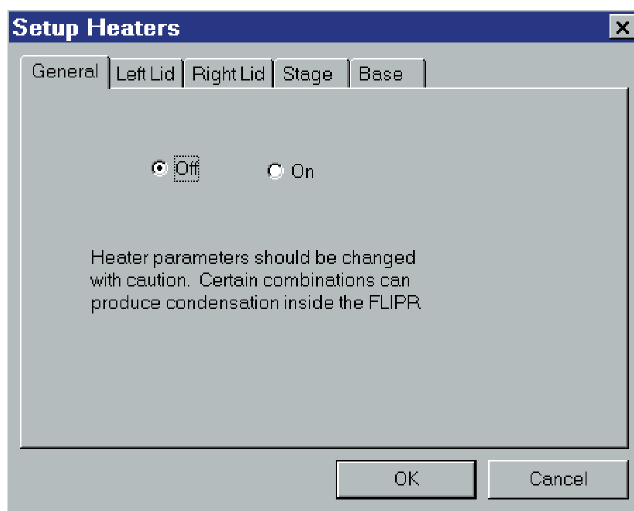


Figure 4-14: The **General** tab of the **Setup Heaters** dialog box.

a) General

This tab allows you to turn the heaters on and off.

b) Left Lid, Right Lid, Stage and Base

These tabs allow you to specify the set point (desired temperature) and upper and lower temperature boundaries at which warnings will be displayed for the corresponding part of the heated stage.

Note: *Contact Molecular Devices before changing any of the temperature parameters in the* **Setup Heaters** *dialog.*

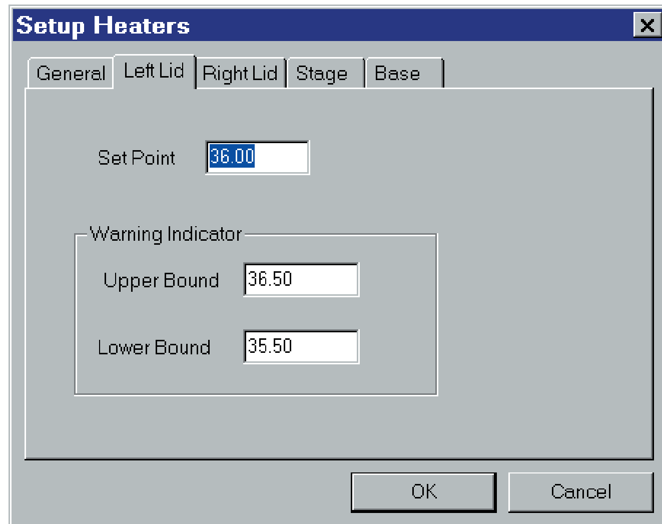


Figure 4-15: The **Left Lid** tab of the **Setup Heaters** dialog box.

4.4.4 Open/Close Main Door

The **Open/Close Main Door** menu item manually opens and closes the FLIPR^{384} door. The door can also be activated by clicking on the door icon at the far right of the FLIPR Tool Bar. The Tool Bar is shown in Figure 4-2 (on page 33). However, the icon is not available during an experiment.

4.4.5 Alignment

Warning: *Alignment should be performed only by trained personnel.*

It is possible to use a either a 96-well or a 384-well plate with a 96-tip head. The FLIPR^{384} System computes the fluorescence signal differently depending on the type of plate being used (384- or 96-well plate). The 384-well plate and the 96-well plate both use the open mask, while the slit-shaped mask is used only with the 96-well plate. The **Alignment** menu item opens the **System Alignment** dialog box (see Figure 4-16). This allows you to specify the type of cell plate and mask (if any) used, and to align the pipettor head with respect to the plate. The alignment procedure is the same for 96- and 384-well formats.

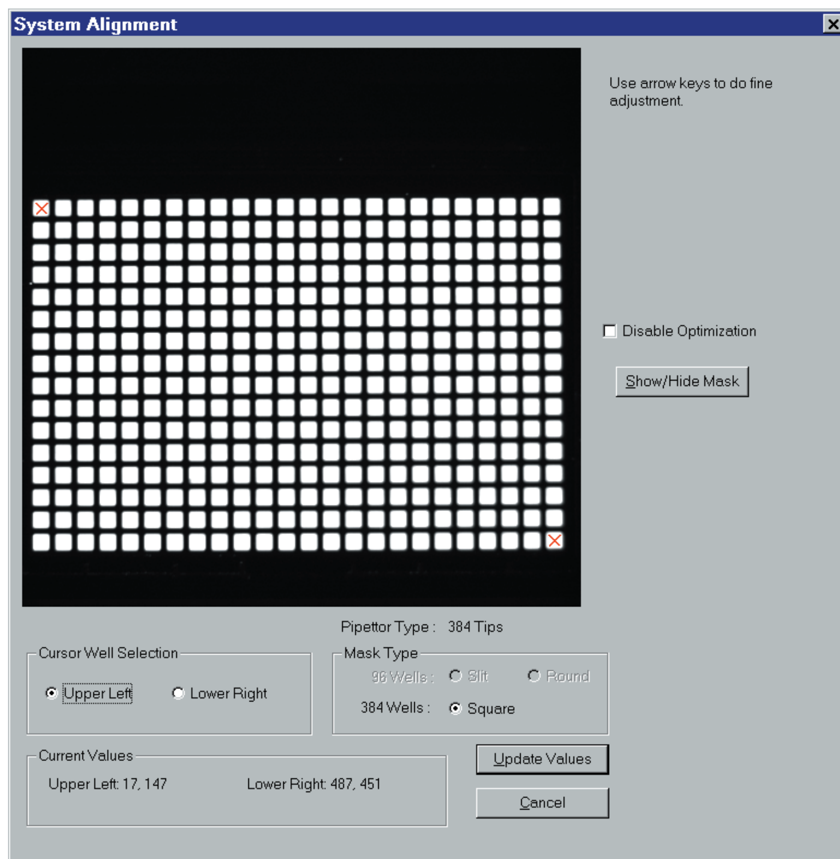


Figure 4-16: The **System Alignment** dialog box.

Even if the cell plate and/or mask are the same as used in the previous assay, the **Alignment** function can be used to optimize the alignment of the camera picture with the well positions. This can potentially decrease the variability across the plate.

Important: *The laser must be on and warmed up in order to perform the alignment procedure.*

Important: *Use the Yellow Plate that matches the cell plate to be analyzed (384- or 96-well).*

Important: *If you want to switch between 96-well and 384-well plates, or switch mask types, you **must** perform the alignment procedure (including changing the* **Mask Type** *settings) and click the* **Update Values** *button to implement the changes. Refer to Chapter 8, "Maintenance," for instructions on the alignment procedure.*

- **Cursor Well Selection**
  This panel 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 located 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, then use the arrow keys to adjust the position of the **X**.
- **Mask Type**
  This panel allows you to specify the type of plate and mask you are using.
- **Disable Optimization**
  This function, when unchecked, optimizes the alignment of the wells with the camera. When this function is checked or disabled, the alignment is set exactly as you clicked in the upper left and lower right wells. We recommend keeping this box unchecked to optimize alignment.
- **Show/Hide Mask**
  This button displays the current alignment, which allows you to determine whether or not further optimization is required.

To enter the new information, click on the **Update Values** button. This will implement the new changes and close the dialog box.

4.4.6 Hardware Setup

Choosing **Hardware** from the **Setup** menu opens the **Hardware Setup** dialog box where you can specify the physical configuration of your FLIPR[384] System (Figure 4-17).

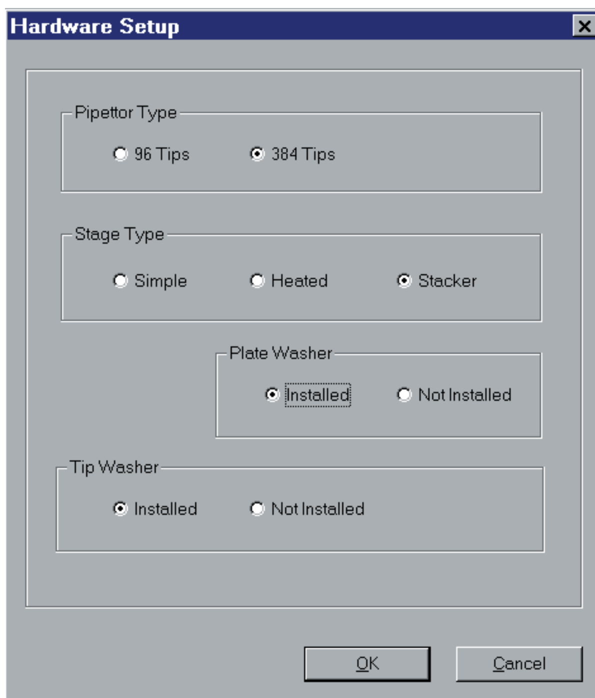


Figure 4-17: The **Hardware Setup** dialog box.

- The **Pipettor Type** panel 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.*

- The **Stage Type** panel specifies which stage is being used. Click the button that corresponds to the appropriate stage.

Note: *If the stage type is changed, the pipettor height settings must be re-checked. See the FLIPR[384] System application note "Adjusting the Pipettor Head Height".*

- The **Plate Washer** panel allows you to specify whether a plate washer (i.e., cell washer) is installed or not.
- The **Tip Washer** panel allows you to specify whether a pipet tip washer is installed or not.

Note: *This setting does not need to be changed if the tip washer is installed. The tip washer is activated in the* **Automation** *tab of the* **Experiment Setup** *dialog (see "Automation" on page 39 for more information).*

After specifying the instrument configuration, click the **OK** button to save the changes and return to the main FLIPR[384] window.

An alert will appear informing you that the application must be rebooted to implement the changes. It gives you the option to continue or cancel the changes (Figure 4-18). You must reboot the application to implement the changes.

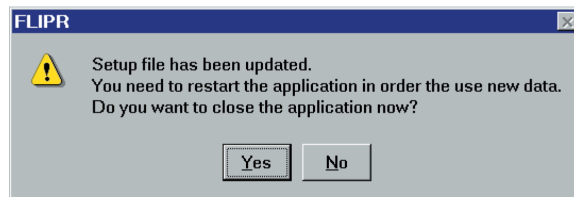


Figure 4-18: The alert triggered when the hardware setup is modified.

4.4.7 Defaults

The **Defaults** menu item has two subsections — **Control Well Layout** and **Graph Setup**. The parameters set in the corresponding dialog boxes are used as defaults when starting an assay. See section 4.7.4, "*Setup*," starting on page 64, for a complete description of the dialog boxes and the functions they contain.

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.

4.5 The Run Menu

The **Run** menu and its **Special Operations** sub-menu are shown in Figure 4-19.

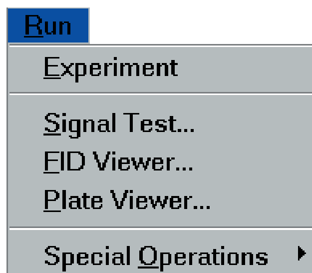


Figure 4-19: The **Run** menu.

4.5.1 Experiment

The **Experiment** menu item initiates an experiment using the parameters defined in the **Setup** dialog box.

Important: *The door closes immediately after any of the* **Run** *menu items are selected.*

4.5.2 Signal Test

Choosing **Signal Test** from the **Run** menu initiates a signal test of the instrument and then displays the results in the **Signal Test** dialog box. An example of **Signal Test** dialog box is shown in Figure 4-20.

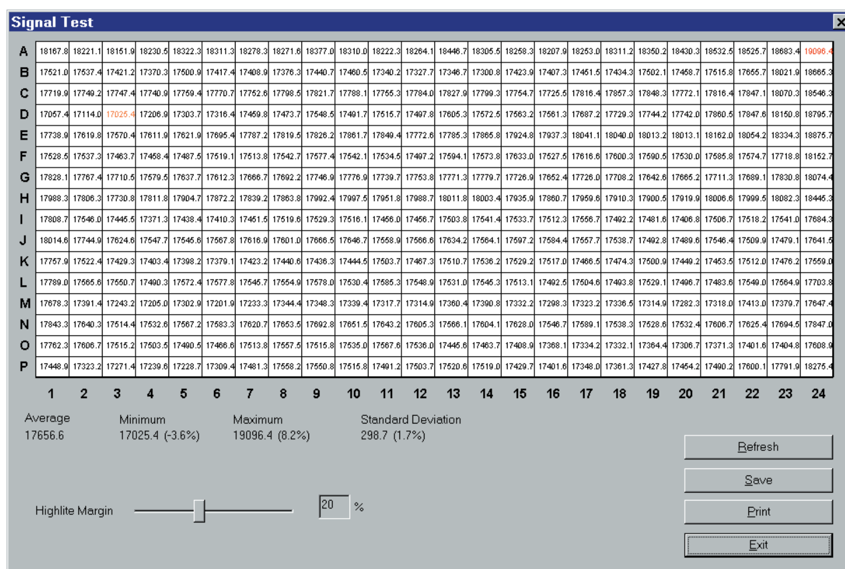


Figure 4-20: The **Signal Test** dialog box.

The signal test has two functions. The first function is an overall system check. The test is typically run once a day using a calibration yellow plate. Make sure the system is turned on and warmed up to ensure that the system is functioning satisfactorily. For a 384-well yellow plate, the exposure time is set to 0.05 sec, the laser power to 600 mW and the camera f/stop to 2. The desirable fluorescence counts for the signal test is 10,000–20,000 counts.

For a 96-well yellow plate, follow the same procedure. The desirable fluorescence intensity is comparable to that of a 384-well yellow plate.

The second function is to check the initial fluorescence of a cell plate just prior to running an assay. The signal test is run using the camera parameters defined in the **Experiment Setup** dialog box. Depending on the range of values obtained, you can choose to either run the experiment or alter the laser, exposure time or camera f/stop settings. Typical signal test values are described in Chapter 5, *"How to Run an Experiment."*

For more information on performing signal tests, refer to Chapter 5, *"How to Run an Experiment."* It is recommended that you print and store copies of the daily signal test for maintenance purposes.

If the measurements are out of the desired ranges, exit the signal test and return to the **Setup Experiment** dialog box. Change the wattage of the laser or the camera's exposure time, as necessary. Return to the signal test again, and check the ranges. Continue this process until the signal is within the ranges. 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. See Chapter 9, *"Troubleshooting"* for more information about saturation error messages.

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

4.5.3 FID Viewer

This menu item opens the **FID Viewer** dialog box (Figure 4-21) allowing you to review every image taken after an experiment. This function is available only for files saved as FID files, not as FWD files. If an experiment was saved in a FID format, the file can be opened and the **FID Viewer** will allow you to play back the sequential images taken during the experiment. You can view the sequence frame-by-frame or sequentially, as a video. The **FID Viewer** data has been compressed from the initial picture which can be visualized using the **Plate Viewer**, therefore, the image will have a lower resolution.

Note: *FID files can be recalled and saved as FWD files. FWD files cannot be saved as FID files.*

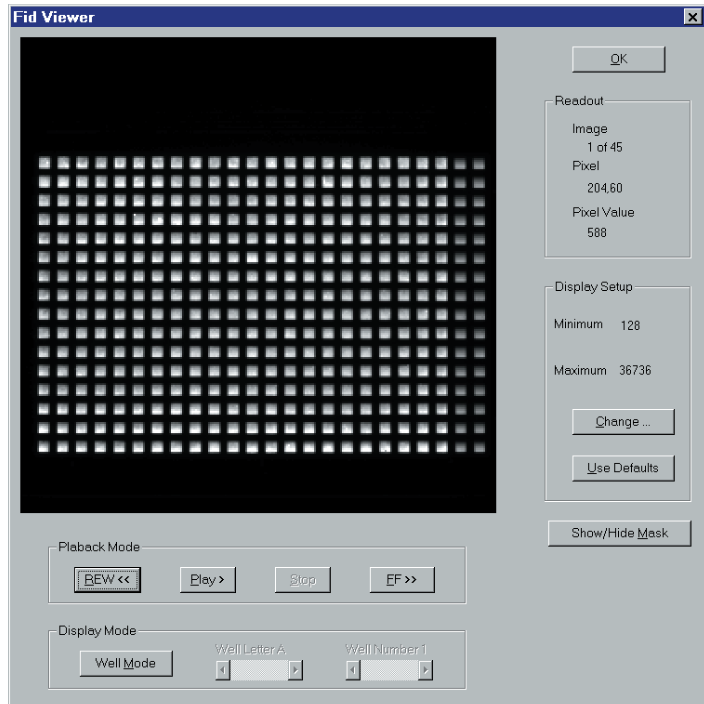


Figure 4-21: The **FID Viewer** dialog box.

- **Display Mode**
  This panel contains options to specify how you want to look at the FID data. You can click on **Full Mode** to view the full plate, or click on **Well Mode** to view data from a single well.
- **Display Setup**
  The **Display Setup** panel allows you to change the minimum and maximum ranges of the fluorescent signal. 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.

- **Use Defaults**
  This button returns the signal max/min ranges to their default values.

The **FID Viewer** can help you diagnose problems. For example, if the cells are blown out upon fluid addition, the **FID Viewer** will display formation of dark "holes" in the bright cell layer after addition of the compounds. If the entire well becomes darker, extracellular dye is likely to be present.

4.5.4 Plate Viewer

This menu item opens the **Plate Viewer** dialog box (Figure 4-22.) This function enables the FLIPR^{384} System to acquire an image of the current plate using the current settings (e.g., exposure length) and then displays it along with the current fluorescence signal. The **Plate Viewer** is the picture equivalent to the fluorescent intensities obtained when running a signal test.

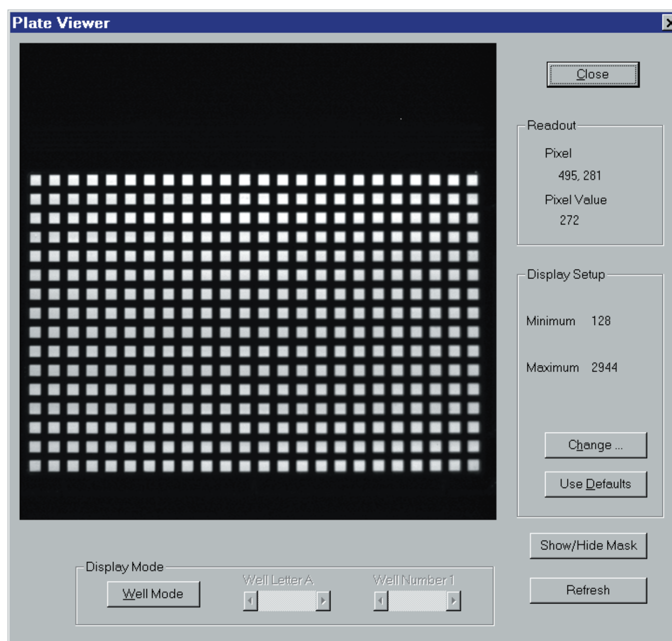


Figure 4-22: The **Plate Viewer** dialog box.

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

Note: *The image obtained using the* **Plate Viewer** *dialog box is not saved as part of a data file.*

- **Display Mode**
  This panel contains options to specify how you want to look at the plate. You can click on **Full Mode** to view the full plate or click on **Well Mode** to view a single well.

- **Display Setup**
  The **Display Setup** panel allows you to change the minimum and maximum ranges of the fluorescent signal. 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.

- **Use Defaults**
  Click on this button to return the signal max/min ranges to their default values.

4.5.5 Special Operations

The **Special Operations** menu contains 4 menu items (Figure 4-23) allowing you to unload and wash tips, prime the tip washer and clear the stacker path. **Unload Tips** unloads the tips to the tip rack or to plate position 4. **Wash Tips** washes the tips using a built-in washing protocol. **Tip Washer Prime** fills the connecting hoses with fluid to ensure that the tip washer is filled with wash fluid prior to a tip wash. **Clear Stacker Path** cycles the stacker and removes any plates to the waste cassette.

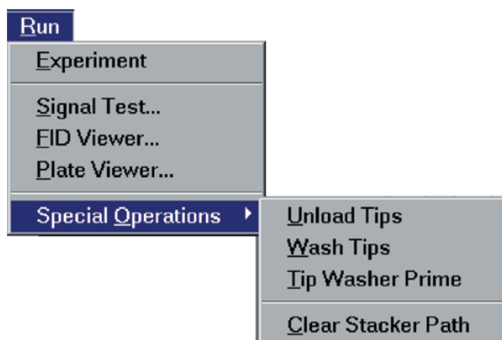


Figure 4-23: The **Special Operations** menu.

4.6 The Stop Menu

The **Stop** menu allows you to terminate an experiment that is in progress. You can also stop an experiment in progress by using the **Stop** icon located on the right side of the Tool Bar (see Figure 4-2, on page 33).

Note: *If you wish to terminate an experimental run after initiating it with the* **Start** *icon, you will have to wait for the pipettor to stop moving before the* **Stop** *icon will be accessible again. Using this feature may freeze the software.*

4.7 The Graphs Menu

The **Graphs** menu is shown in Figure 4-24.

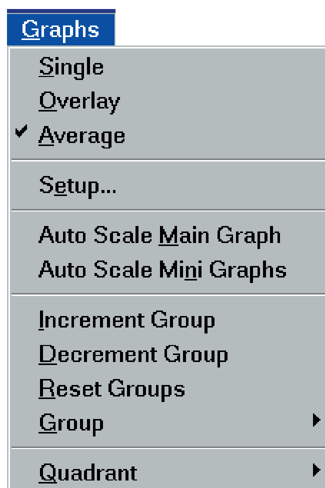


Figure 4-24: The **Graphs** menu.

4.7.1 Single

Selecting this menu item will display the data from a single well (i.e., one mini graph) in the main graph. You can choose the well to be displayed by clicking the corresponding mini graph with the left mouse button.

4.7.2 Overlay

The **Overlay** menu item displays multiple wells on the main graph. This function displays all of the selected individual mini graphs. You can select a series of mini graphs by dragging the cursor over the desired wells while holding down the left mouse button. You can deselect wells by clicking on the well(s) with the right mouse button.

4.7.3 Average

This menu item shows the average graph for a selected group of wells. You can create as many as 24 different groups of wells. To create a group, highlight the wells you want averaged. A group number is automatically assigned. Press the up arrow on the keyboard to move to the next group number, then use the mouse to highlight the wells for the next group.

4.7.4 Setup

The **Setup** menu item opens the **Graph Setup** dialog box (Figure 4-25). The dialog has three tabs of options labelled **Graph Setup**, **Control Well Layout**, and **Group Names**.

Note: *The* **Graph Setup** *dialog box affecting only the default graph settings can be accessed by choosing* **Default fiGraph Setup** *from the* **Setup** *menu* *(see "Defaults" on page 56).*

a) Graph Setup

The options chosen in the **Graph Setup** tab of the **Graph Setup** dialog box will override any graph defaults set using the **Setu**p menu. The tab contains four panels — **Scale**, **Processing**, **Grid** and **Group Display** (Figure 4-25).

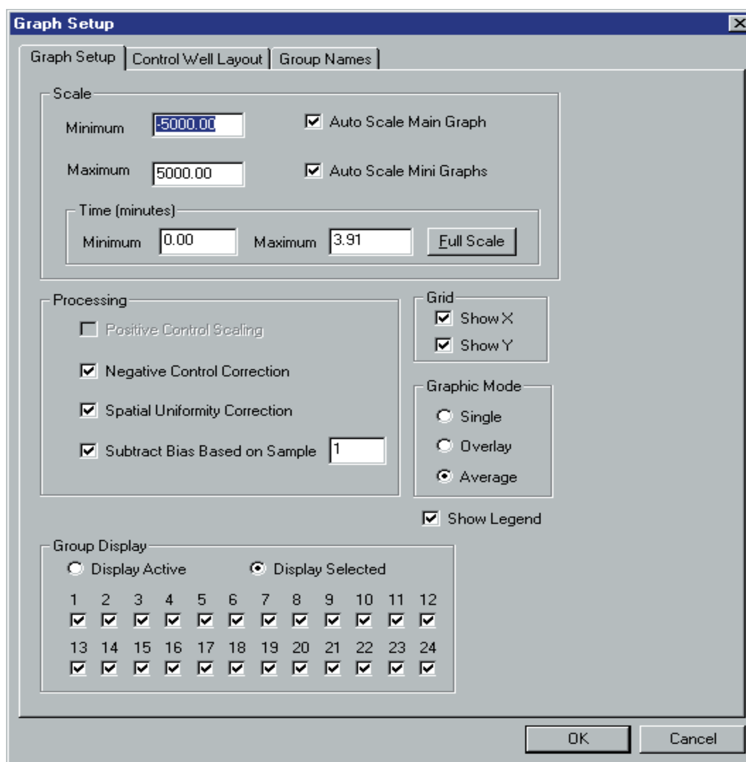


Figure 4-25: The **Graph Setup** tab of the **Graph Setup** dialog box.

Scale

This panel allows you to choose how the Y-axis (fluorescence counts) and the X-axis (time) will be scaled. Most users choose to auto-scale the main graph (which displays only highlighted 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**

  The default value for this parameter is -5000, but it can be changed to any desired value. This parameter is implemented if the **Autoscale** box is not checked.

- **Maximum**
  The default value for this parameter is +5000, but it can be changed to any desired value. This parameter is implemented only if the **Autoscale** box is not checked.
- **Autoscale Main Graph**
  This parameter 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.
- **Autoscale Mini Graphs**
  This parameter 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.

Processing

This panel contains four options that allow you to manipulate the signal data in different ways.

Note: *Additional information about how the FLIPR^{384} Control software processes data is provided in Appendix D, "FLIPR Systems Data Processing Algorithms."*

- **Positive Control Scaling**
  This function 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**
  This function 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 wells. This function provides a good correction for signal drift and artifacts.

- **Spatial Uniformity Correction**
  This function 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 cell number, type and dye-loading conditions are constant throughout the plate.

Note: *In addition to spatial uniformity correction, a built-in software intensity correction feature takes the laser illumination correction into account. This protocol is given in Appendix E, "Optical Correction Protocol."*

- **Subtract Bias Based on Sample**
  This function subtracts the fluorescence value measured at a selected sample (reading) from all of 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 ("0"). Select the sample to subtract by checking **Single** on the **Graphs** menu, then moving the cursor arrow to the graph and clicking 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 (see Figure 4-1) will display the position of the cursor (i.e., which sample it's in), the time, bias value and the delta.

Note: *The cursor 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 the grid lines for the fluorescence (Y) axis in the main graph.

Graphic Mode

This panel controls the display of graphic mode in the main graph.

- **Single**
  Check this option to display the data from a single well in the main graph.
- **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.

Group Display

This panel controls the display of the groups you selected. The FLIPR^{384} System control software allows data from individual wells to be averaged together in groups 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 **Single** is checked on the **Graphs** menu, this option will graph the highlighted (red) mini graph on the main graph. When **Overlay** is checked on the

Graphs menu, this option will graph all of the highlighted (red) mini graphs on the main graph concurrently. When **Average** is checked on the **Graphs** menu, this option will graph the average of the selected group on the main graph.

- **Display Selected**
  Check this option, and then check the box(es) corresponding to the group(s) you want to display in the main graph. If **Average** is checked on the **Graphs** menu, multiple averaged groups will be displayed concurrently on the main graph. If **Single** or **Overlay** is checked on the **Graphs** menu, the display will be the same as when the **Display Active** option is selected (i.e., data from one or more individual wells will be displayed).

Show Legend
When checked, this causes the main graph's legend to be displayed.

b) Control Well Layout

This tab of the **Graph Setup** dialog box allows you to designate the positive and negative control wells (see Figure 4-26). A single click on the selected well will mark it as a positive control (typically maximum response to a concentration of a given agonist), while a double-click will mark 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.

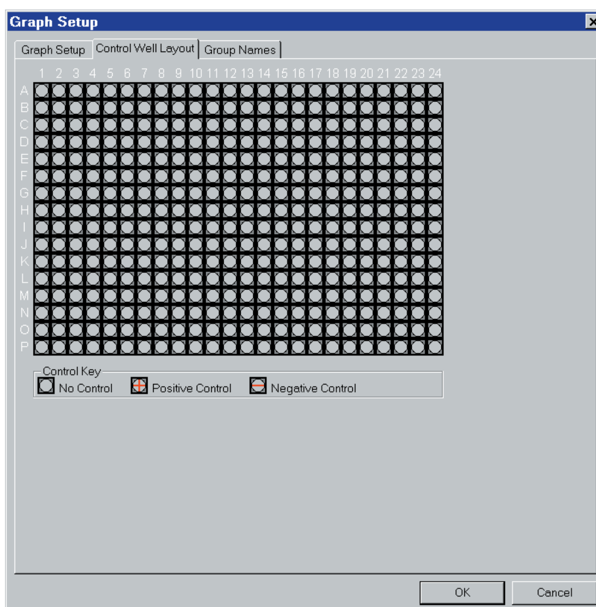


Figure 4-26: The **Control Well Layout** tab of the **Graph Setup** dialog box.

c) Group Names

This tab of the **Graph Setup** dialog box allows you to name the groups according to the compound added (Figure 4-27).

Note: *The* **Group Names** *tab is not available in the* **Graph Setup** *dialog box accessed by selecting* **Setup → Defaults → Graph Setup**.

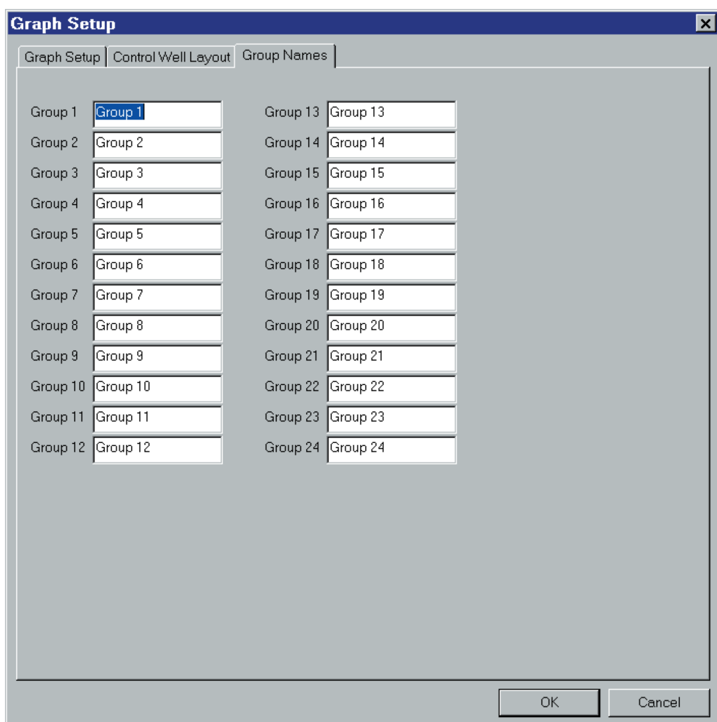


Figure 4-27: The **Group Names** tab of the **Graph Setup** dialog box.

4.7.5 Autoscale Main Graph

This parameter 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. This function can also be accessed from the **Graph Setup** tab of the **Graph Setup** dialog box.

4.7.6 Autoscale Mini Graphs

This parameter 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. This function can also be accessed from the **Graph Setup** tab of the **Graph Setup** dialog box.

4.7.7 Increment Group

When the **Display Selected** option has been chosen in the **Graph Setup** tab of the **Graph Setup** dialog and **Averaged** has been checked in the **Graphs** menu, 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 (equivalent to pressing the up arrow on the keyboard).

4.7.8 Decrement Group

When the **Display Selected** option has been chosen in the **Graph Setup** tab of the **Graph Setup** dialog and **Averaged** has been checked in the **Graphs** menu, this selects the previous 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 (equivalent to pressing the up arrow on the keyboard).

4.7.9 Reset Groups

This menu item allows you to erase all of the previously defined groups and start over.

4.7.10 Group

This menu item assigns a specific group number to a selected group of wells and allows you to choose which of the groups to highlight in the graphs.

4.7.11 Quadrant

The **Quadrant** menu is shown in Figure 4-28. This menu allows you to choose an individual quadrant and display its data in the mini graph window. To display all four quadrants, select **All**. See Figure 4-29 for an illustration of how the quadrants are arranged in a 384-well plate.

Note: *This option is available only when using a 384-well cell plate.*

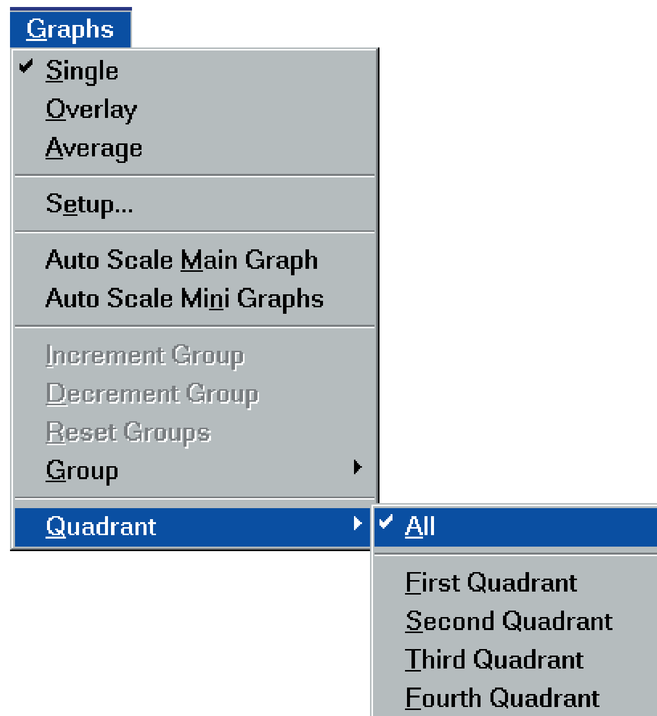


Figure 4-28: The **Quadrant** menu.

There are four quadrants to choose from (1–4, see Figure 4-29):

- **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.

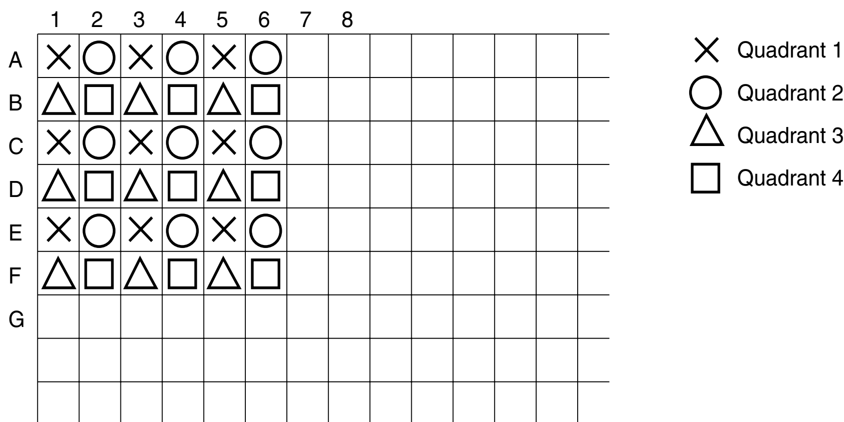


Figure 4-29: Quadrants in a 384-well plate.

4.8 The Window Menu

This menu provides you with three different ways to organize the data on the screen (see Figure 4-30).

The **Cascade** and **Tile** options refer to the way that multiple open data files are laid out on the screen. If the **Cascade** option is selected, the files are overlapped and staggered with the title bars visible. If the **Tile** option is selected, the screen is divided into as many segments as there are files. 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.

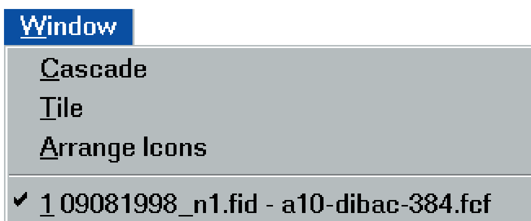


Figure 4-30: The **Window** menu.

At the bottom of the **Window** menu is a list of all of the open data files along with the template file that they were run with.

4.9 The View Menu

The **View** menu is shown in Figure 4-31. This menu allows you to select the combination of screens that you want displayed simul-

taneously. All of the menu items, with the exceptions of **Main Graph** and **Mini Graph**, are toggle switches that turn the display of the selected item on and off.

Note: *The* **View** *menu will not display the* **Experimental Setup Parameters**, **Main Graph** *or* **Mini Graph** *screens unless a control file has been loaded.*

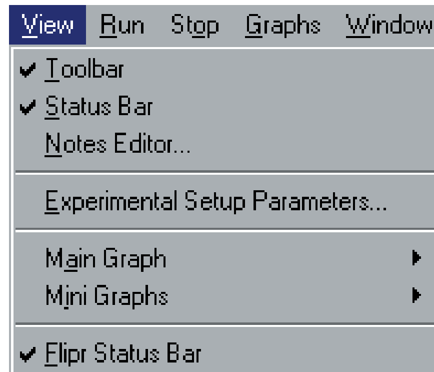


Figure 4-31: The **View** menu.

4.9.1 Toolbar

This menu item allows you to toggle display the Tool Bar (Figure 4-32) on and off. The Tool Bar is displayed when there is a "check" next to the menu item.

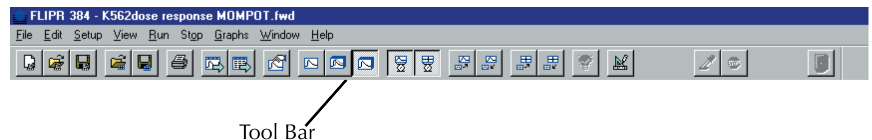
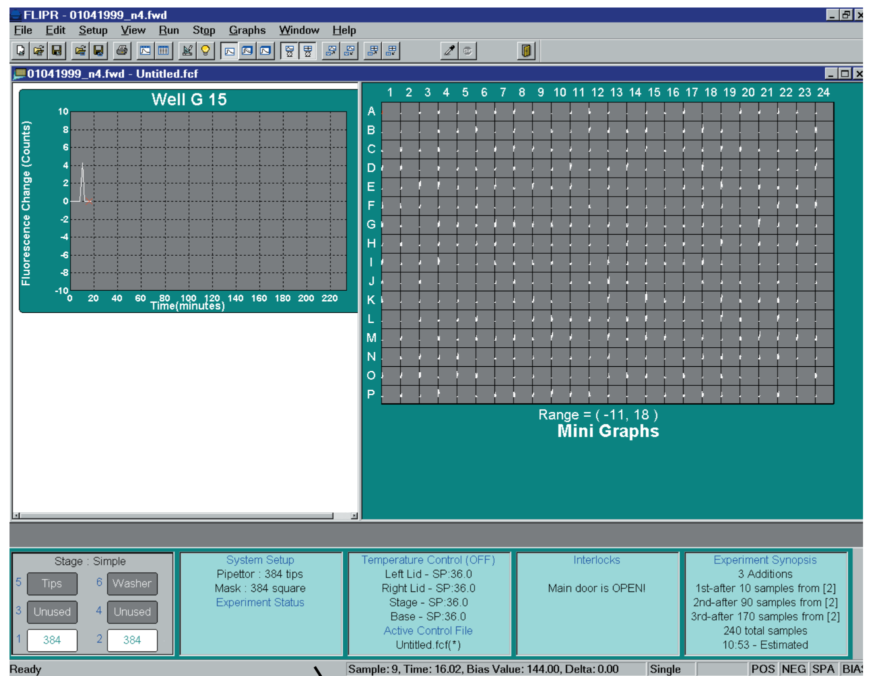


Figure 4-32: The FLIPR^{384} Tool Bar.

4.9.2 Status Bar

This menu item allows you to toggle display the Status Bar on and off. The Status Bar is located at the bottom of the screen and is displayed when there is a check next to the menu item.



Status Bar

Figure 4-33: The Status Bar.

The Status Bar displays the name of the active group. An active group is any graph processing functions being used, such as subtract bias ("**BIAS**"), negative ("**NEG**") and positive ("**POS**") control wells and the spatial uniformity correction ("**SPA**"). If you are using the cursor to step through the timepoints on the graph, in order to select a sample for bias subtraction, the Status Bar will display the position of the cursor (i.e., which sample it's in), the time, bias value and the delta (see "*Subtract Bias Based on Sample*" on page 67).

4.9.3 Notes Editor

This menu item allows you to enter notes that will be saved with the data file. The notes will be printed if the **Print Selections** item of the **File** menu is checked. See the **File** menu description starting on page 80.

4.9.4 Experimental Setup Parameters...

This menu item opens the **Experimental Setup Parameters** dialog box, displaying all parameters and settings for the control file currently running (Figure 4-34). Only information for control files (FCF files) is displayed — no FWD or FID file information.

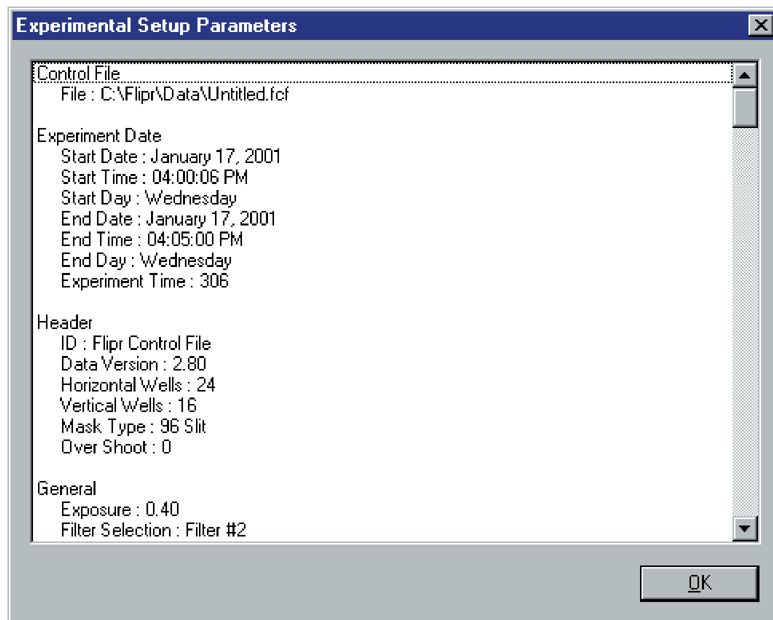


Figure 4-34: The **Experimental Setup Parameters** dialog box.

4.9.5 Main Graph

The **Main Graph** menu (Figure 4-35) allows you to “zoom in” to increase the main graph’s size or “zoom out” to decrease it. To return the graph to its previous size (the size before the last modification), select **Reset**.

Note: *This function can be used repeatedly to obtain a desired display size.*

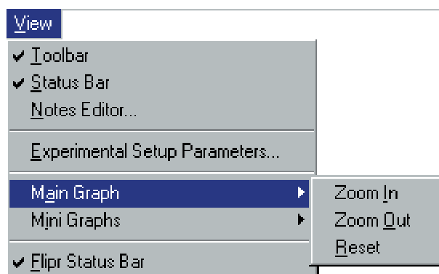


Figure 4-35: The **Main Graph** menu.

4.9.6 Mini Graphs

The **Mini Graphs** menu allows you to "zoom in" to increase the main graph's size or "zoom out" to decrease it. To return the graph to its previous size (before the last modification), select **Reset**.

Note: *This function can be used repeatedly to obtain a desired display size.*

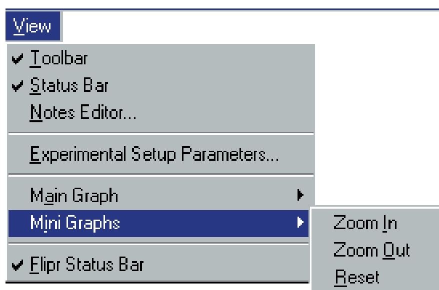
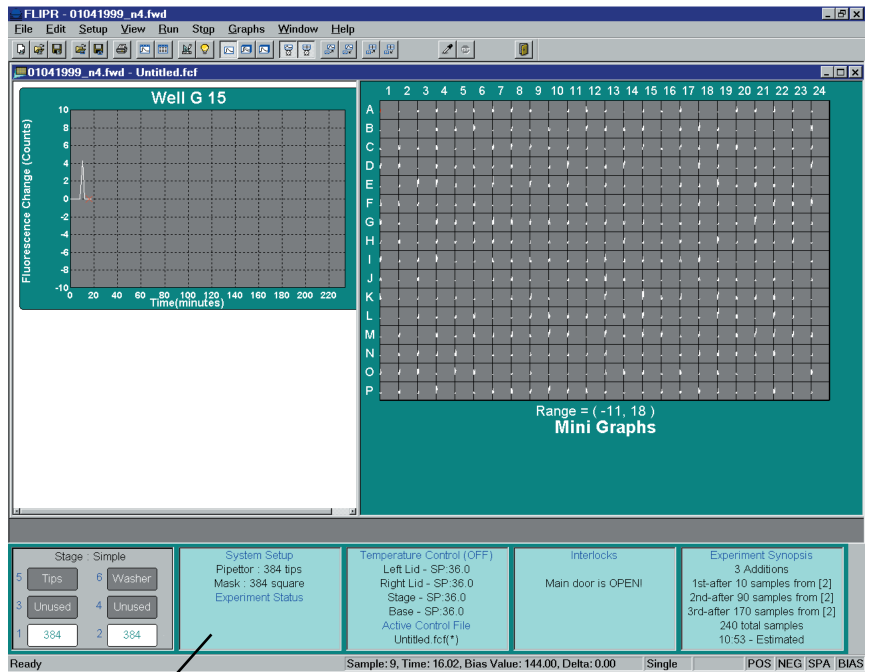


Figure 4-36: The **Mini Graphs** menu.

4.9.7 FLIPR Status Bar

This menu item allows you to toggle display the FLIPR Status Bar on and off. The FLIPR Status Bar is located below the graphs and above the Status Bar. It provides a protocol summary and temperature information. It is displayed when there is a check next to the menu item.



FLIPR Status Bar

Figure 4-37: The FLIPR Status Bar.

4.10 The File Menu

The **File** menu is shown in Figure 4-38. Each of the menu items is described in the following subsections.

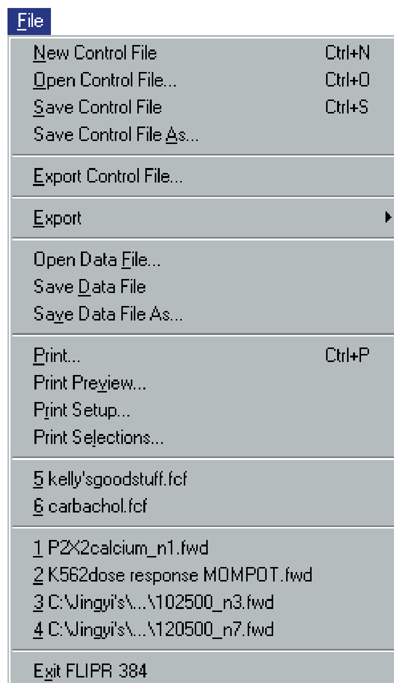


Figure 4-38: The **File** menu.

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

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

4.10.1 New Control File

This menu item opens a new control file with the default settings.

4.10.2 Open Control File...

This menu item opens an existing control file.

4.10.3 Save Control File

This menu item saves updates to the currently opened control file.

4.10.4 Save Control File As...

This menu item allows you to save modifications performed on an existing control file as a new control file with a new name.

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 to set all experiment parameters.

4.10.5 Export

The **Export** menu item allows you to save FLIPR^{384} 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's **Automation** tab (see "*Export Time Sequence/Statistics/Control File after Experiment*" on page 40). This menu contains four options — **Time Sequence**, **Statistics**, **Main Graph** and **Mini Graph** (Figure 4-39).

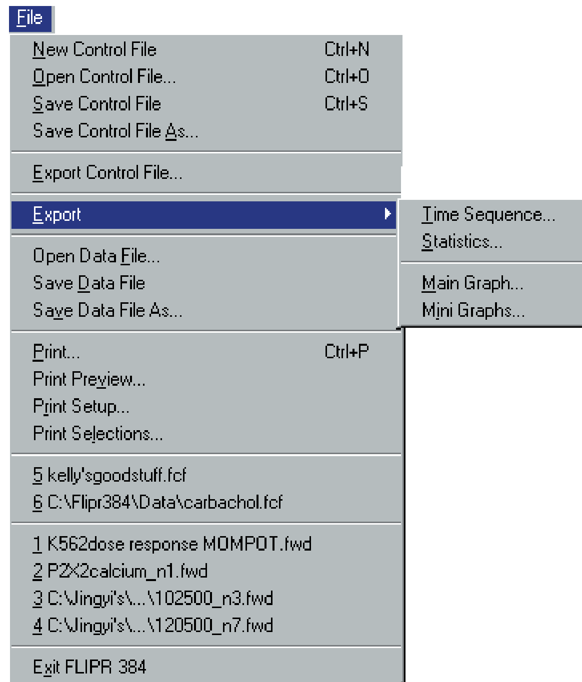


Figure 4-39: The **Export** menu.

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 data point per well.

4.10.6 Time Sequence

This menu item opens the **Export Time Sequence** dialog box, which contains five separate panels (Figure 4-40) — **Export Data From**, **File Naming**, **Data Format**, **Export Time Sequence**, and **From Quadrant.**

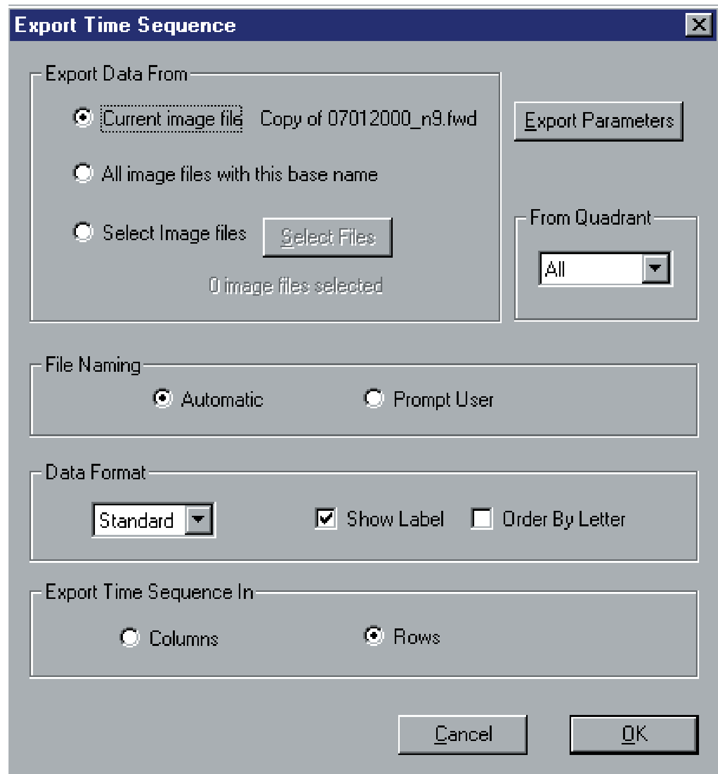


Figure 4-40: The **Export Time Sequence** dialog box.

- **Export Data From...**
  This panel allows you to export an image file from any of three different sources - the current image file, all image files with a selected base name or a specifically-chosen image file. In some cases, multiple data files may be open. In these instances, the **Current image file** option refers to the most recently opened file (i.e., the file displayed most recently on the screen). The **All image files with this base name** option refers to a group of files saved using the same base name (either full date or user-defined). This option gathers data from all of the files into one large ASCII file, which can be convenient for data analysis. The **Select image files**

option allows you to open a list of image files and select one or more to be exported.

Important: *If the* **All image files with this base name** *option is selected for export, all of the files having the base name must be present, or none of the files will be exported. The alternative is to choose the* **Select image files** *option.*

- **File Naming**
  Here you can select either 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: basename_nX.squ, where X represents the number of the FID file. When selecting all image files with the same base name, the name will be as follows: Basename_mult.squ.
- **Data Format**
  The default data format is **Standard, 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 replicates are laid out in the plate. Make sure the replicate wells are consecutive in the spreadsheet to ensure easy data handling.
- **Export Time Sequence**
  This panel of options allows you to export the data in columns or rows. The choice depends on the type of spreadsheet program you are using.

Important: *If exporting data for analysis using Microsoft Excel, note that Excel can accept only 256 columns of data. Data from a 384-well plate must be exported in rows.*

- **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. This 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 (Figure 4-41) which enables you to select experimental parameters to export with the data. The choices in this dialog box can be saved in a template file for future use.

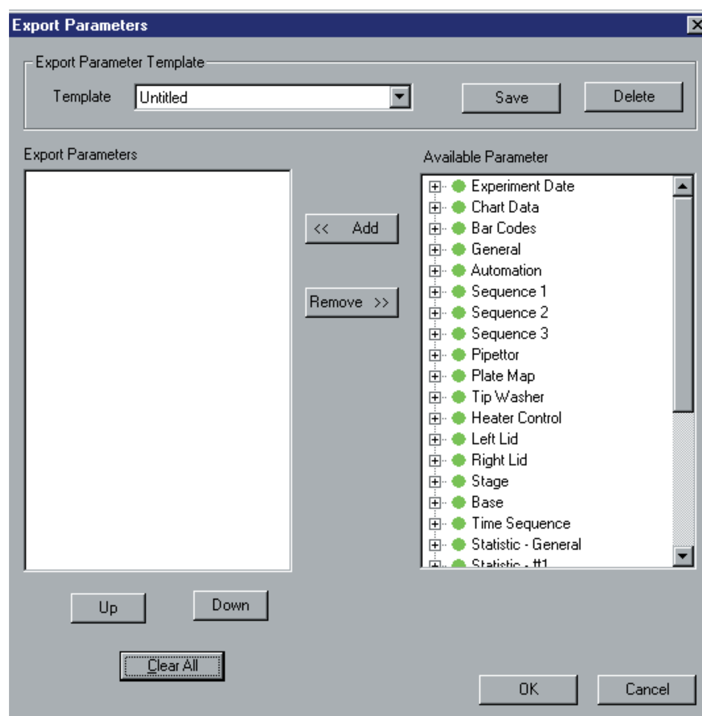


Figure 4-41: The **Export Parameters** dialog box.

4.10.7 Statistics

This menu item opens the **Export Statistics** dialog box containing four tabs of options — **General**, **Statistic #1**, **Statistic #2** and **Statistic #3** (see Figure 4-42).

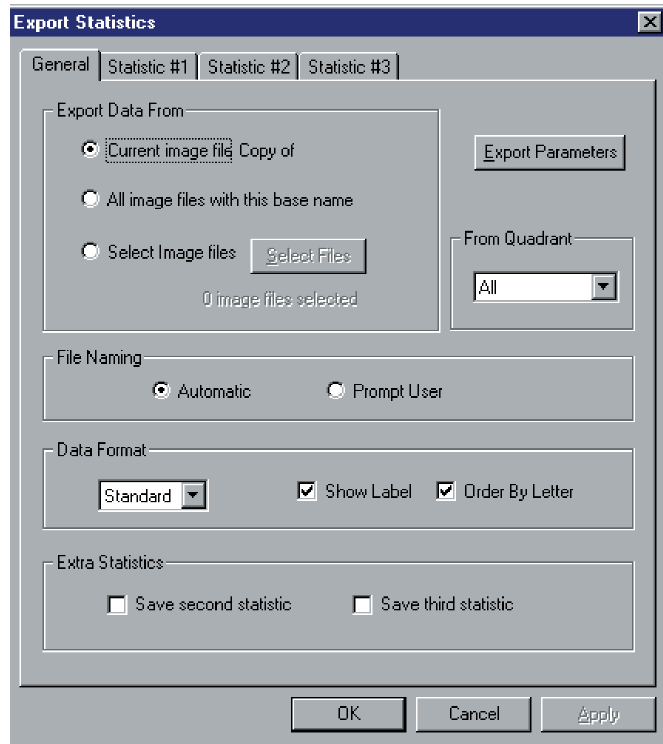


Figure 4-42: The **General** tab of the **Export Statistics** dialog box.

a) General

This tab contains options very similar to those in the **Export Time Sequence** dialog box (see Figure 4-40). The **Export Data From**, **File Naming**, **Data Format**, and **From Quadrant** panels work in a similar fashion as described previously. However, in exporting statistics files, the file naming is as follows. When the **Current image file** option is chosen, the file naming pattern will be Basename_nX.stat. If the **All image files with this base name** option is chosen, the file naming pattern will be Basename_mult.stat.

The **Save second statistic** and **Save third statistic** options allow you to save second and third statistics from the same data. The file name of the second statistic saved will be Basename_nX.stat2.stat when the **Current image file** option is selected, or Basename_mult.stat2.stat when the **All image files with this base name** option is selected. Similarly, the file name of the third statistic saved will be Basename_nX.stat3.stat when the **Current image file** option is selected, or Basename_mult.stat3.stat when the **All image files with this base name** option is selected.

b) Statistic #1/Statistic #2/Statistic #3

These tabs are identical to each other (see Figure 4-43). The **Compute** panel contains options that allow you to compute the data in any of five different ways:

- **Sum**
  The numerical sum of the fluorescence counts of the selected images. Also called area under the curve.
- **Average**
  The numerical average of the fluorescence counts of the selected samples or readings.
- **Min**
  The lowest fluorescence count (a single number) of all of the images selected.
- **Max**
  The highest fluorescence count (a single number) of all of the images selected.
- **Max–Min**
  The result of subtracting the minimum fluorescence count (single number) from the maximum fluorescence count (single number).

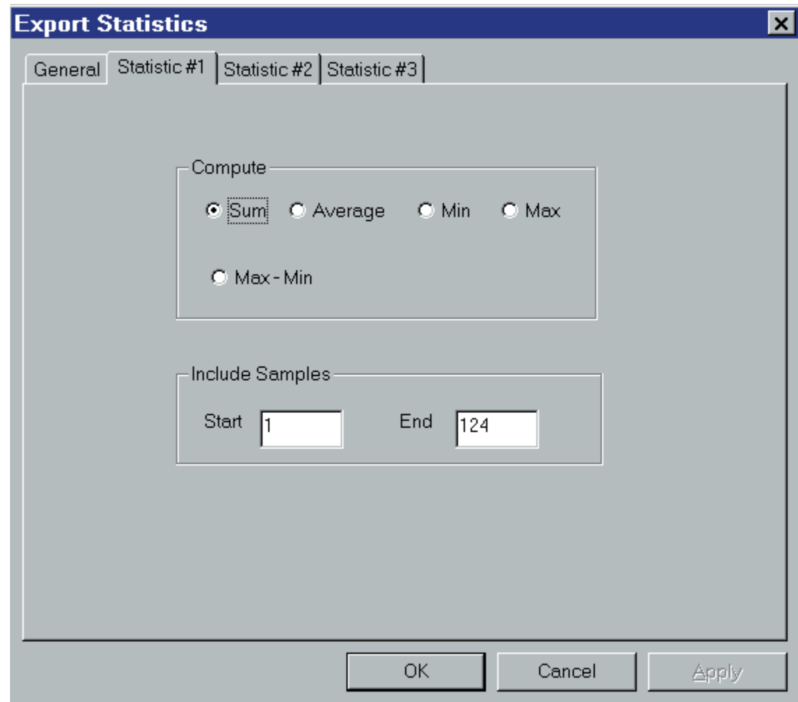


Figure 4-43: The **Statistic #1** tab of the **Export Statistics** dialog box.

The **Include Samples** panel allows you to specify the samples at which the exporting begins and ends.

Caution: *Make sure you have checked the "***Save second statistic***" and/or "***Save third statistic***" boxes in the* **General** *tab of the* **Export Statistics** *dialog box, if you want to save the second/third statistic!*

4.10.8 Main Graph

Opens a dialog box that allows you to specify a location in which to export the main graph (Figure 4-44). The graph is exported as a .WMF format file that can be imported into Word, Excel, etc.

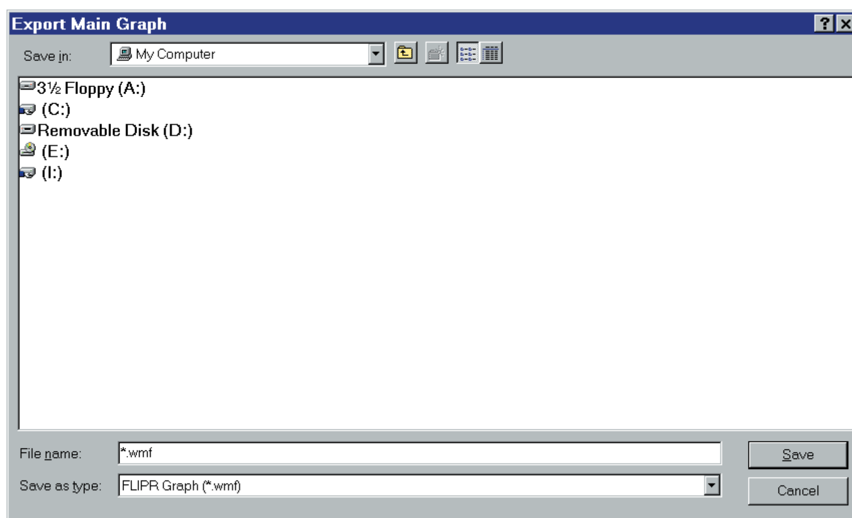


Figure 4-44: The **Export Main Graph** dialog box.

4.10.9 Mini Graphs

This menu item works the same way as **Export Main Graph**, but it allows you to export the mini graphs into Word, Paint, etc.

The third portion of the **File** menu deals with handling data files.

4.10.10 Open Data File

Opens an archived FID or FWD data file. You may view multiple data files simultaneously. The open data files are listed in the fourth section of the **File** menu.

4.10.11Save Data File

Saves the data file with the automatic name assigned by the software (using the base name, the date or user-defined name).

Note that 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.

4.10.12 Save Data File As...

Saves the data file with a name other than the name automatically assigned by the software.

4.10.13 Print

Prints whatever has been selected in the **Print Selection** dialog box (see Figure 4-45).

4.10.14 Print Preview

Allows you to preview items before printing them.

4.10.15 Print Setup

Allows you to define the printer settings, paper size and paper orientation.

4.10.16 Print Selections

This menu item (Figure 4-45) opens the **Print Selections** dialog box. It allows you to choose what you would like to print (i.e., the main graph, mini graphs, notes, experiment setup dialog box, or graph and control well setup dialog boxes).

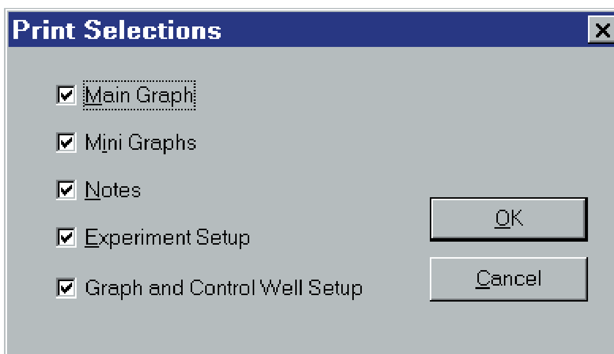


Figure 4-45: The **Print Selections** dialog box.

4.10.17 The File Lists in the File Menu

The fourth section of the **File** menu lists the four most recent control (FCF) files used. The fifth section of the **File** menu lists the four most recent data (FID or FWD) files opened.

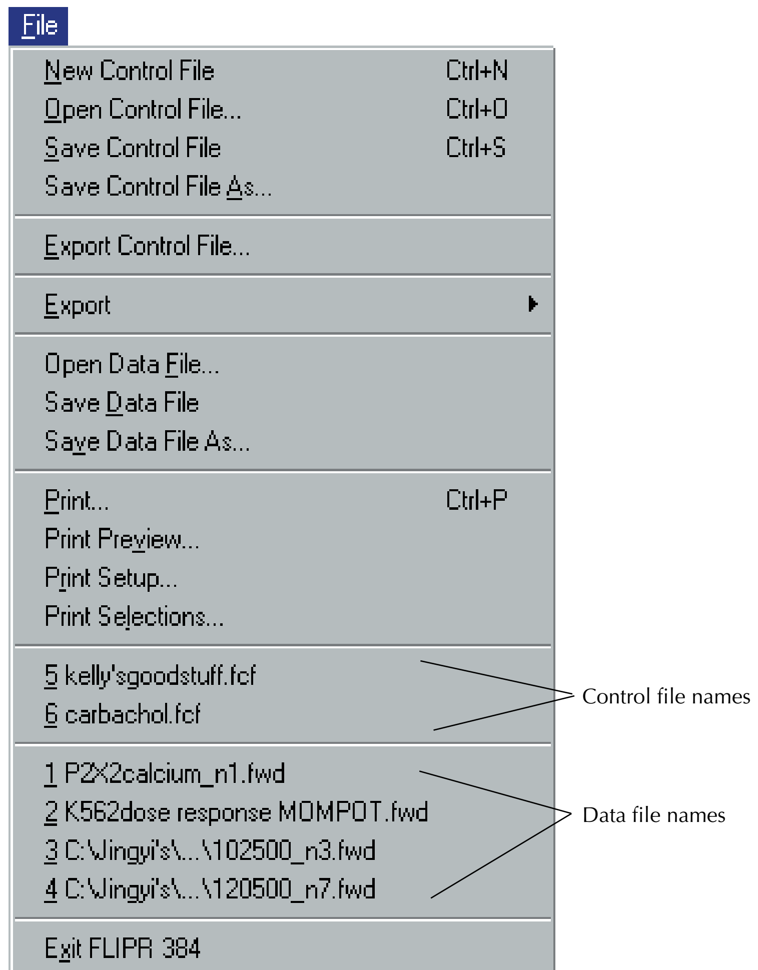


Figure 4-46: The fourth and fifth sections of **File** menu contain lists of the control and data files, respectively.

4.10.18 Exit

Allows you to exit the program. You should exit the program before powering down the FLIPR^{384} System.

Chapter 5
How to Run an Experiment

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5.1 Introduction

This chapter provides a starting point for setting up and running a fluorescence assay on the FLIPR^{384} System.

The first two sections explain the preparation of cells and the general principle of a fluorescence assay on the FLIPR^{384} System. The two subsequent sections describe standard protocols for measuring intracellular calcium flux and intracellular membrane potential.

Appendix A provides a list of consumables and other supplies necessary to run FLIPR^{384} assays using 384- or 96-well plates.

5.2 Preparing Cells for an Experiment

The main optical feature of the FLIPR^{384} 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.

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.

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.

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. The basic steps of the assay are:

1) Plate the cells
2) Dye-load the cells
3) Assay the cells in the FLIPR system

Note: *Flat-bottom, clear-wall tissue culture plates can also be used with the FLIPR Calcium Assay Kit.*

It is necessary to optimize the cell seeding density so that a uniform, 80% to 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.

Note: *The specific protocols for intracellular calcium and membrane potential assays are outlined in sections 5.4 and 5.6, respectively.*

5.3 Running an Experiment

This section provides an overview of the steps involved in a fluorescence assay on the FLIPR^{384} System. Specific protocols for calcium flux or membrane potential assays are provided in subsequent sections.

5.3.1 Turn On the FLIPR^{384} System

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 either one and enter the modifications in the **Hardware Setup** dialog box (see Chapter 4, *"Software Overview,"* for details on the **Hardware Setup** dialog box).

Note: *Please contact Molecular Devices if you need to change the pipettor head.*

If temperature regulation is desired, 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.

5.3.2 Run a Yellow Plate Test

Once a day, 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.*

a) 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's f/stop to f/2. Check by looking at the camera lens through the right side door.
- Set the exposure time to 0.05 second (**General** tab of the **Experiment Setup** dialog box, see Figure 4-5 on page 36).

b) Yellow Plate Test Parameters for a 96-well Plate

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

- 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's f/stop to f/2. Check by looking at the camera lens through the right side door.
- Set the exposure time to 0.05 second. (**General** tab of the **Experiment Setup** dialog box, see Figure 4-5 on page 36).

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

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 between 10,000–20,000 counts. If the test results are outside of the range of acceptable values, perform the laser alignment procedure.

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.

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 "cell 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.*

5.3.3 Dye Load the Cells

In order to be visualized, the cells are incubated in the presence of a fluorescent dye. The dye can be calcium sensitive for intrac-

ellular calcium flux assays, or membrane potential sensitive for membrane potential assays. The dye loading step typically lasts one hour either in a 37 °C incubator or at room temperature.

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. A 60-minute 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 minutes 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.*

5.3.4 Compound Plate Preparation

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.

Polypropylene plates are recommended to limit sticking of the compounds 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.

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.

Intracellular calcium and membrane potential measurements need to be performed quickly to capture rapid cellular kinetics. Therefore, it is necessary to pipet the compounds into the cell plate rapidly and to effectively mix the fluids in the wells. However, 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 minutes in a dry incubator (15 minutes in a heating block) to warm the plates to equilibrium at 37° C. Please refer to Sections 5.4 and 5.6 for the specific protocols for intracellular calcium and membrane potential assays.

5.3.5 Set Up an Assay Protocol

The easiest way to use the FLIPR^{384} System control software is to make different types of template 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 template files, open the relevant FCF file by choosing **Open** from the **File** menu and selecting the appropriate template file from the dialog box. Alternatively, the bottom portion of the **File** menu lists the last four FCF template files that have been used. This allows you to simply choose the template file from the menu.

a) Create a Template File

To create a new template file, define each of the following parameters (see Chapter 4, *"Software Overview,"* for details on using the dialog boxes mentioned in the following steps):

1) In the **Experiment Setup** dialog box (accessed by choosing **Experiment** from the **Setup** menu), define the camera exposure time, the type of plate used and all pipettor parameters including tip washing (for parameters specific to calcium flux and membrane potential assays, see Sections 5.4 and 5.6, respectively).

   Note: *The* **Fluid Addition** *dialog boxes will not be available until the plate map has been defined.*

   Note: *Make sure that the tip washer reservoir has wash fluid, if tip washing is selected.*

2) In the **File Names** dialog box (accessed by choosing **File Names** from the **Setup** menu), define the destinations of the saved and exported files.

3) In the **Graph Setup** dialog box (accessed by choosing **Graph Setup** from the **Defaults** pull-down menu in the **Setup** menu), define the graph parameters such as the scaling, type of display and data correction.

The above parameters constitute the bare minimum for defining a template file. You can save it by selecting **Save As** from the **File** menu and save the file as an FCF file. Additional parameters, such as export and print functions, can be defined and saved under the same template name at the time the template file is created, or later on.

5.3.6 Starting a 384-well Plate Experiment

1) Place the cell and compound plates on the stage.

2) Place a tip rack in the tip pick-up position.

3) Start with the laser power, camera f-stop, and exposure time settings shown in Table 5-1. These settings are the most frequently used to measure basal fluorescence signal.

Settings	Membrane Potential Assay	Calcium Assay
Laser power (W)	0.600–1.20	0.600–1.20
Camera f/stop	f/4.0	f/2.0
Exposure time (sec.)	0.4	0.4
Filter	Filter #2	Filter #1

Table 5-1: FLIPR System setup parameters for measuring basal fluorescence signal.

4) 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 fluorescent 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 following three parameters can be adjusted.

- **Laser Power**
  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 2.000 W on the FLIPR^{384} system.

- **Exposure Time**
  If the basal fluorescence signal is too high, the exposure time can be decreased, to a minimum of 0.05 second. If the basal fluorescence signal is too low, the exposure time can be increased, but the sampling interval will have to be increased to a minimum:

exposure time + 0.6 second (it takes 0.6 seconds to integrate data).

- **Camera f-stop**
  Increasing the camera f-stop number decreases the opening of the aperture. If the basal fluorescence signal is too high, increase the f-stop from 2.0 to 2.8 or more. If the basal fluorescence signal is too low, the laser power may be increased. f/2 is the largest aperture opening setting for the FLIPR system.

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

5) Set the fluid dispensing parameters using the FLIPR control software.

   - **Pipettor Height**
     For 96-well pipettor, after the pipettor picks up fluid from the compound plate, it draws a small bubble of air 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. 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, 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.

- **Fluid Dispensing Speed**
  The default pipettor dispensing speed is 50 µL/sec for 96-well plates and 20 µL/sec for 384-well plates. Table 5-2 provides the limits for the fluid dispensing speed for both 96- and 384-well plate formats.

	96-well Plate	384-well Plate
Slow dispensing speed (µL/sec.) Weakly adherent cells or non-adherent cells	10–40	5–10
Fast dispensing speed (µL/sec.) Strongly adherent cells	50–80	15–20

Table 5-2: Recommended fluid dispensing speeds for the calcium assay.

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 to dislodge cells from the well.

6) Check the status bar to verify that the experiment synopsis is accurate and that the appropriate FCF file is being used.

7) Click the **Run** button 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.

8) At the end of the assay, the main door opens and the instrument is available for the next assay.

Warning: *If multiple users are operating the instrument at the same time, be sure that other users are far from the door before clicking* **Run**.

5.4 Protocol for Intracellular Calcium Assays Using the FLIPR Calcium Assay Kit

5.4.1 Materials

1) Calcium Assay Kit (see consumables list in Appendix A)
2) Clear, flat-bottom, black-wall or clear-wall 384- or 96-well plates (see consumables list in Appendix A)
3) Clear, polypropylene compound plate (see consumables list in Appendix A)
4) FLIPR pipette tips, 96-well or 384-well (see consumables list in Appendix A).
5) Cells of choice in suspension
6) Test compounds
7) Growth medium
8) Incubator (5% CO_2, 37 °C)
9) Centrifuge
10) Pipettor and sterile tips suitable for use with microplates
11) Probenecid in powder form
12) 1 N NaOH

Fluorescent Dye

The contents of the FLIPR Calcium Assay Kit are optimized to give a robust result when used on the FLIPR system according to the protocol. 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.

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

Anion 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 duration of the dye loading step should be kept to a minimum.

5.4.2 Cell Preparation

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 minutes (**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% to 90% confluent cell monolayer, we recommend seeding densities as shown in Table 5-3.

	96-well Plate	**384-well Plate**
Adherent cells ($x10^4$cells/well)	2–10	0.5–3
Non–adherent cells ($x10^4$cells/well)	10–50	2.5–10
Growth medium volume (µL)	100	25

Table 5-3: Recommended cell densities for calcium assays on FLIPR.

5.4.3 Dye Loading

a) 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.

1) To prepare the 1X Reagent Buffer, pipette 10 mL of 10X Reagent Buffer (Component B in Calcium Assay Kit) and dilute 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 the Troubleshooting Guide (see page 115).*

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. Do not store frozen aliquots of Loading Buffer with probenecid, and always add fresh probenecid the day of the experiment.

b) Loading Cells Using Loading Buffer

4) 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).

5) 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.*

5.4.4 Running the Calcium Assay on the FLIPR System

After incubation, transfer the plates directly to FLIPR and begin the calcium assay. Recommended experimental setup parameters are given in Table 5-4.

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

Table 5-4: Experimental parameters for calcium assays on FLIPR.

5.5 Troubleshooting Intracellular Calcium Assays

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 have 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 Table 5-5, 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

Table 5-5: Suggested dye concentrations to eliminate dye dilution.

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. Table 5-6 is provided as a guideline.

	Loading Buffer		Compound	
Plate	**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

Table 5-6: Alternate concentrations and volumes of the Dye Loading Buffer.

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 Assays

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.

5.6 Protocol for Membrane Potential Assays Using the FLIPR Membrane Potential Assay Kit

5.6.1 Materials

1) FLIPR® System with the 540–590 nm BP FLIPR Emission Filter installed (Molecular Devices Corporation)
2) Membrane Potential Assay Kit (Molecular Devices Corporation, R-8034)
3) Clear, flat-bottom, black- or clear-wall 384- or 96-well plates (see consumables list in Appendix A, *"Consumables Used With the FLIPR System"*)
4) Clear, polypropylene compound plates (see consumables list in Appendix A)
5) FLIPR pipette tips, 96-well or 384-well (see consumables list in Appendix A).
6) Cells of choice in suspension
7) Growth medium
8) Test compounds

9) Incubator (5% CO_2, 37 °C)

10) Centrifuge

11) Pipettor and sterile tips suitable for use with microplates

12) 1 N NaOH

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.

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

5.6.2 Cell Preparation

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 minutes ***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% to 90% confluent cell monolayer,

it is recommended the cells be seeded at densities shown in Table 5-7.

	96-well Plate	**384-well Plate**
Adherent cells (x10^4 cells/well)	2–10	0.5–3
Non-adherent cells (x10^4cells/well)	10–50	2.5–10
Growth medium volume (µL)	100	25

Table 5-7: Recommended cell densities for membrane potential assays on FLIPR.

5.6.3 Dye Loading

a) 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.

1) To prepare the 1X Reagent Buffer, dilute 10 mL of 10X Reagent Buffer (Component B in the Membrane Potential Assay Kit) to 100 mL with distilled water, adjusting 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.*

b) Loading Cells Using Loading Buffer

3) 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).

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

5.6.4 Running the Membrane Potential Assay on FLIPR

A filter must be installed in the Filter #2 position of the FLIPR system prior to running the assay. 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. Remove one ring by unscrewing in a counter-clockwise direction. Carefully place the 540–590 BP FLIPR Emission Filter in the #2 location and screw the ring back in place with the notches facing outward. Place the filter holder in its correct position in FLIPR.

After incubation with the Loading Buffer, transfer the plates directly to FLIPR and begin the assay. Recommended experimental setup parameters are given in Table 5-8.

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

Table 5-8: Experimental parameters for membrane potential assays on FLIPR.

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.*

5.7 Troubleshooting Membrane Potential Assays

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 on 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 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.

5.8 Optimizing an Assay

The majority of the FLIPR^{384} assay optimization is related to cell treatment prior to and during the assay. Please refer to *"Running an Experiment"* on page 96 for of all the details on assay conditions.

5.8.1 Cell Treatment Conditions To Check/Optimize Before an Assay

- Cell passage number
- Cell growth conditions, for example confluency in flasks and/ or in wells
- Expression induction time for transfected cells

5.8.2 Conditions To Check/Optimize During an Assay

a) Cell Seeding

- 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

b) Dye Loading

- 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)

c) Compound Plates

- 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

d) Cell Washing

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

e) Preparing the Instrument

- 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

Chapter 6
The Plate Stacker

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6.4 Running Experiments Using the Plate Stacker Accessory 130

6.1 Introduction

The plate stacker (see Figure 6-1) is an accessory that can be integrated with the FLIPR^{384} 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^{384} 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^{384} System.

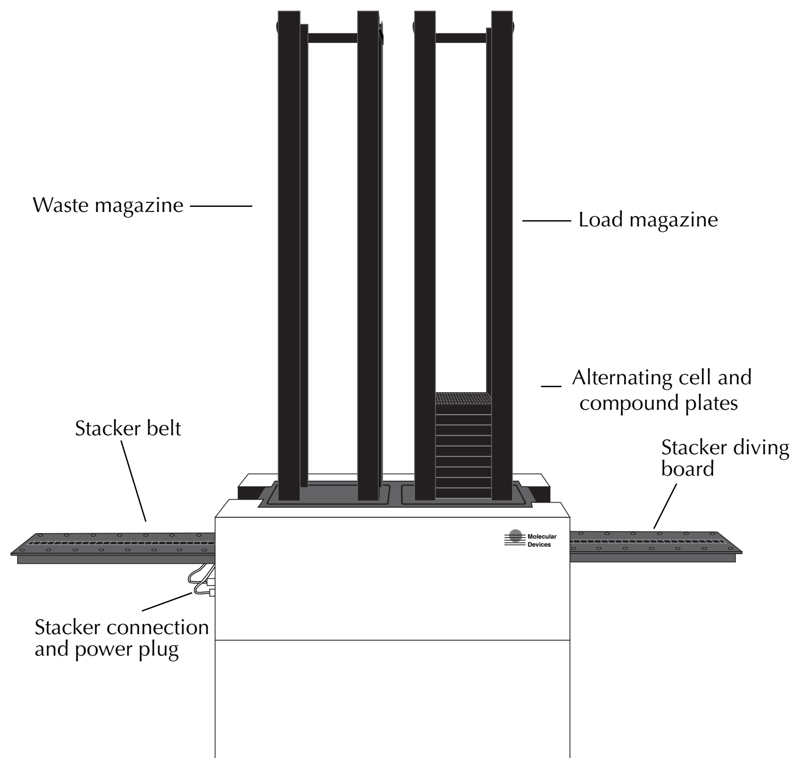

Figure 6-1: Diagram of the plate stacker.

6.2 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 (see Figure 6-1). During installation, the service representative will adjust the stacker position and air pressure for optimal operation.

6.2.1 The Stacker Unit

1) Turn on the FLIPR^{384} 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^{384} instrument door. Removing this side plate allows the FLIPR^{384} 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.

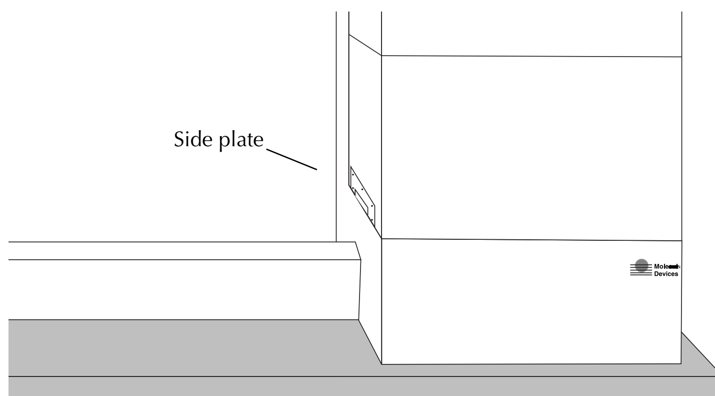

Figure 6-2: Detail of the side plate.

3) Position the plate stacker on the laser cover, which is located on the left side of the FLIPR^{384} instrument. The stacker belt should be parallel to the laser. Make sure the two reference pins located on the FLIPR^{384} platform fit into the two locating holes in the stacker belt (see Figure 6-3).

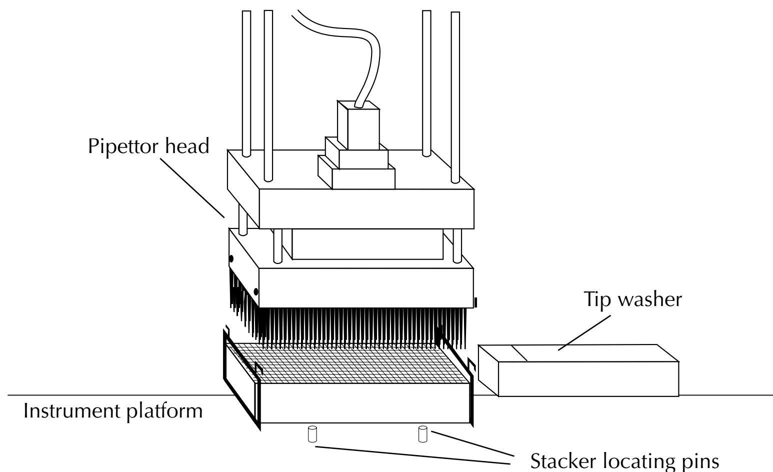

Figure 6-3: Diagram of the stacker belt locating pins on the instrument platform.

4) Connect the RS232 cable between the FLIPR^{384} 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^{384} control software is launched and the stacker is in power mode, the stacker initialization will take place first. This process takes approximately 35 seconds and consists of testing belt movements and magazine pistons.

6.2.2 Stacker Assay Platform

1) Place the stacker stage on the FLIPR^{384} system platform (see Figure 6-4). Make sure that the stage locating pins fit in the platform holes.

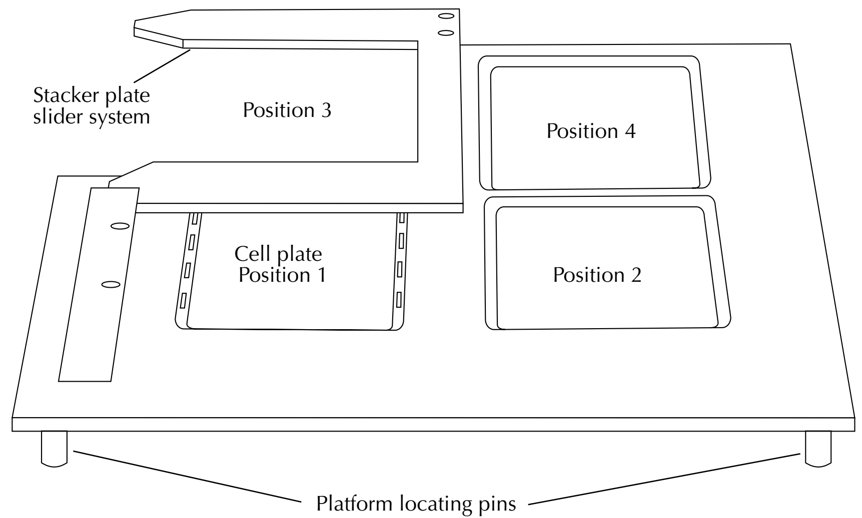

Figure 6-4: Diagram of the stacker stage.

2) Connect the stacker stage to the FLIPR^{384} instrument by inserting the circular and square connectors into the matching plugs located above the stage.

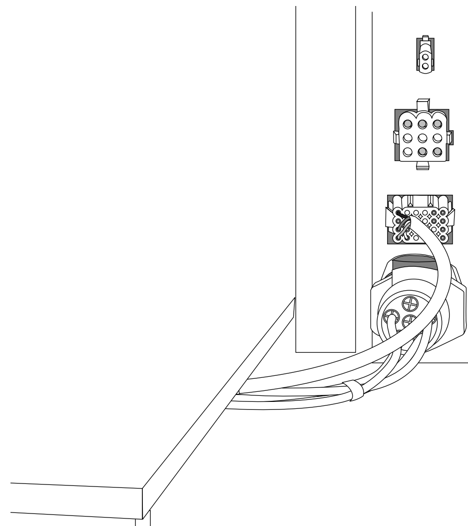

Figure 6-5: Connecting the stacker stage to the FLIPR^{384} system.

Note: *The simple and heated stages are not compatible with the plate stacker.*

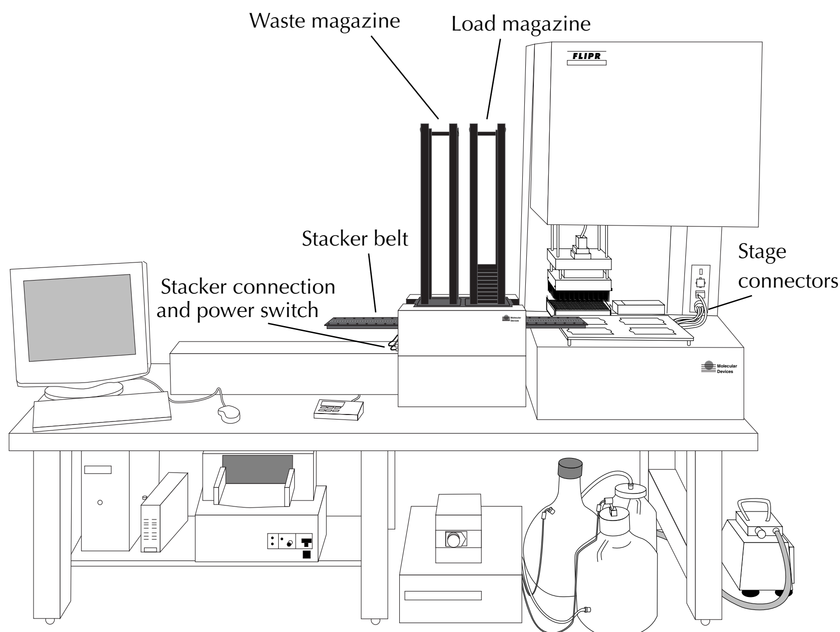

Figure 6-6: Diagram of the FLIPR^{384} system with the plate stacker installed.

6.2.3 Removing the Stacker

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.

6.3 Software Setup for the Plate Stacker Accessory

6.3.1 Setup File Modification

1) Select **Hardware** from the **Setup** menu to open the **Hardware Setup** dialog box (Figure 6-7).

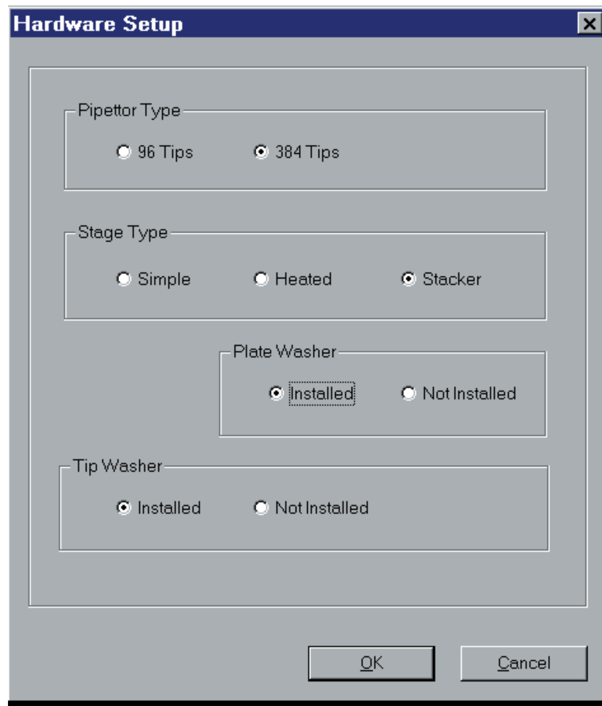

Figure 6-7: The **Hardware Setup** dialog box

2) In the **Stage Type** panel, select **Stacker**. Click the **OK** button in the **Hardware Setup** dialog. An alert will appear, informing you that the application must be rebooted to implement the changes and gives you the option to continue or cancel (Figure 6-8). Click **Yes** to close the application and implement the change.

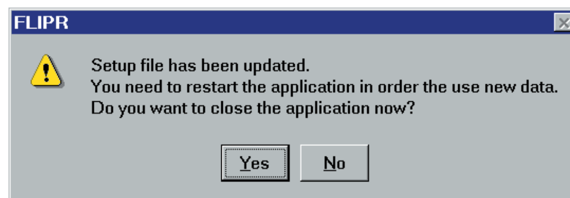

Figure 6-8: The alert triggered when the hardware setup is modified

3) Restart the FLIPR^{384} control software.

4) When using the stacker accessory, the **Load Cell Plate...** option in the **Automation** tab of the **Experiment Setup** dialog is automatically selected (see Figure 6-9). If desired, you can also select the **Load Sample Plate** option.

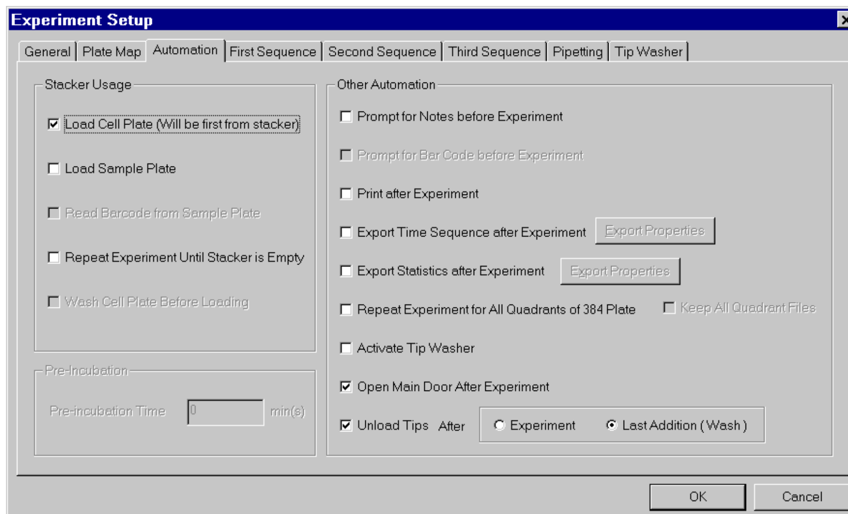

Figure 6-9: The **Automation** tab of the **Experiment Setup** dialog.

6.3.2 Running a Yellow Plate Test with the Stacker

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 I yellow plate (which has an attached lid) on the stacker.*

1) To run a signal test or plate view using the yellow test plate, select **Signal Test** or **Plate Viewer** from the **Run** menu. To check the alignment using the yellow plate, choose **Alignment** from the **Setup** menu.

2) A dialog box will appear, asking “Do you want to load a signal plate?”

3) Click **Yes** and place the test plate in the load magazine. The plate will be transferred to the “1” reading position. If you click **No**, the plate will not be moved.

4) After the selected function is complete, a dialog box will appear, asking "Do you want to return the signal plate?" If you select **Yes**, the plate will be returned to the waste magazine. If you select **No**, another function (signal test, plate viewer or alignment) may be performed using the test plate.

Note: *The yellow plate must be transferred to the read position using the plate stacker.*

6.3.3 Setting Up an Experiment File Using the Plate Stacker Accessory

1) Select **Experiment** from the **Setup** menu to open the **Experiment Setup** dialog. The **Automation** tab of the dialog contains options for programming the plate stacker (see Figure 6-10).

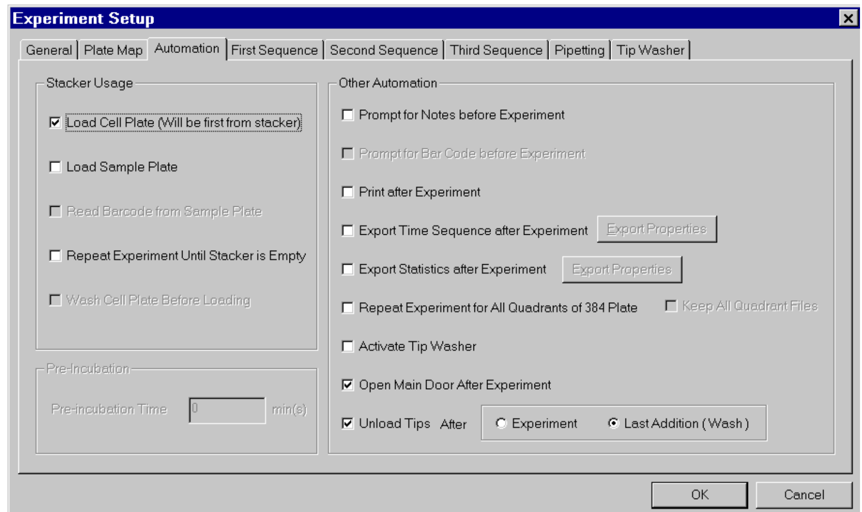

Figure 6-10: The **Automation** tab of the **Experiment Setup** dialog.

2) Five options are available on the left side of the **Automation** tab:

- **Load Cell Plate**
  When the **Hardware Setup** has been configured to use the stacker, this option is automatically selected. The cell plates are always placed in stage position "1".

- **Load Sample Plate**
  To run an assay using a compound plate, select this option. The stacker always places the compound plate in stage position "3". This function is optional. You can either load compound plates using the stacker, or you can manually add compound plates in stage positions "2" and/or "4".
- **Read Bar Code From Sample Plate**
  To assign a bar code name to the data file, select this option. The date is no longer used in the file name, and the bar code will be used as the file name. Export files will also have the same bar code name.

Note: *If the* **Read Bar Code** *option is selected and the plates do not have a bar code, the default file name will be: badbarcode_date_nx.*

- **Repeat Experiment Until Stacker Is Empty**
  When the entire series of cell and compound plates is ready to be run, this option may be selected.
- **Wash Cell Plate Before Loading**
  This option affects the use of the cell washer (see Chapter 7, *"The Plate Washer,"* for additional details) and is only available when a plate washer is installed on the FLIPR^{384} System.

3) The **Activate Tip Washer** option is also located in the **Automation** tab (refer to Figure 6-10). By default, the tips are not changed between plates for the entire plate series, but they may be washed. The tips may also be washed between fluid additions within the same experiment (see Chapter 4, *"Software Overview"* for additional details on this feature).

4) The **Plate Map** tab of the **Experiment Setup** dialog is used to define where the compound plates are located (refer to Figure 6-11). Make sure that position "3" is selected for the fluid addition if the compound plate will be coming from the stacker.

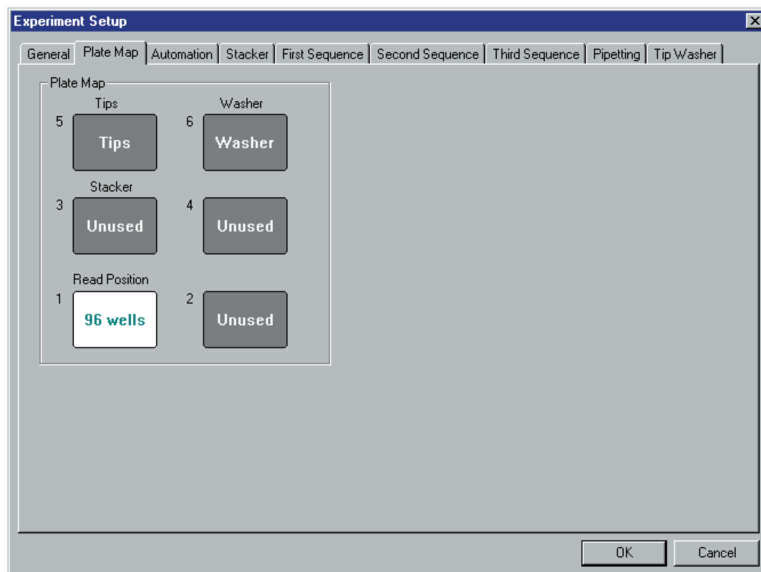

Figure 6-11: The **Plate Map** tab of the **Experiment Setup** dialog box

5) Define the rest of the experimental parameters, such as the volumes added, etc.

Note: *Positions "2" and "4" can be used for compound addition, yet the plates are not changeable during an experimental set. Deep-well compound plates may be a suitable option for stage positions "2" and "4".*

6.4 Running Experiments Using the Plate Stacker Accessory

6.4.1 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.*

6.4.2 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. A dialog box will appear, asking "Do you want to load a signal plate?" Clicking **Yes** activates the signal test for your cell plate. 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 can not be performed before each assay. A signal test can be performed only for the first cell plate.

6.4.3 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.

Chapter 7
The Plate Washer

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7.1 Introduction

This chapter contains information on setting up and operating the FLIPR^{384} and FLIPR^{96} systems plate washer. Use it as a reference for daily operation and as a troubleshooting guide.

7.1.1 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.

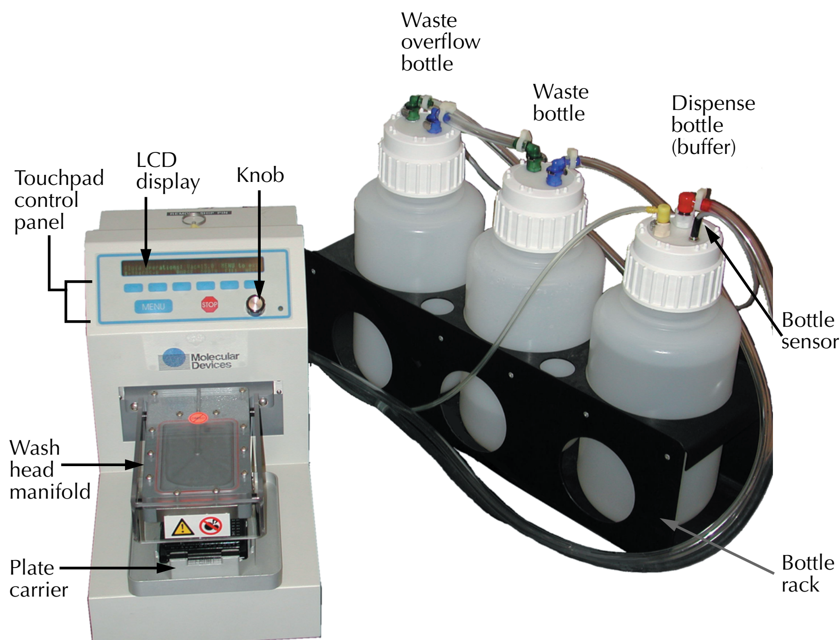

Figure 7-1: The 96/384 plate washer.

7.1.2 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 Table .

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.
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.

Table 7-1: Plate washer features and their functions.

7.2 Setting Up the Plate Washer

7.2.1 Plate Washer Components

The following items are included in the plate washer shipment:

- 1 User Manual addendum
- 1 Base unit with wash head
- 1 Plate carrier
- 1 Bottle rack assembly
- 1 Buffer container with cap and fittings, 4L
- 2 Waste containers with cap and fittings, 4L
- 1 Bottle rack
- 2 Power cords
- 1 Vacuum pump
- Base unit to electrical source
- Base unit to vacuum pump
- 1 RS232 interface cable
- 1 Safety shield
- 1 Maintenance kit

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.

7.2.2 Unpacking the Base Unit

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.*

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.*

7.2.3 Unpacking the Bottle Rack Assembly

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.*

7.2.4 Plate Washer Installation

a) Tubing Connections

1) Reconnect the tubing connections to the appropriate bottles. The collars are color coded for easy identification and installation. Refer to Table for the color legend and Figure 7-2 for proper setup.

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

Table 7-2: Tubing connection colors.

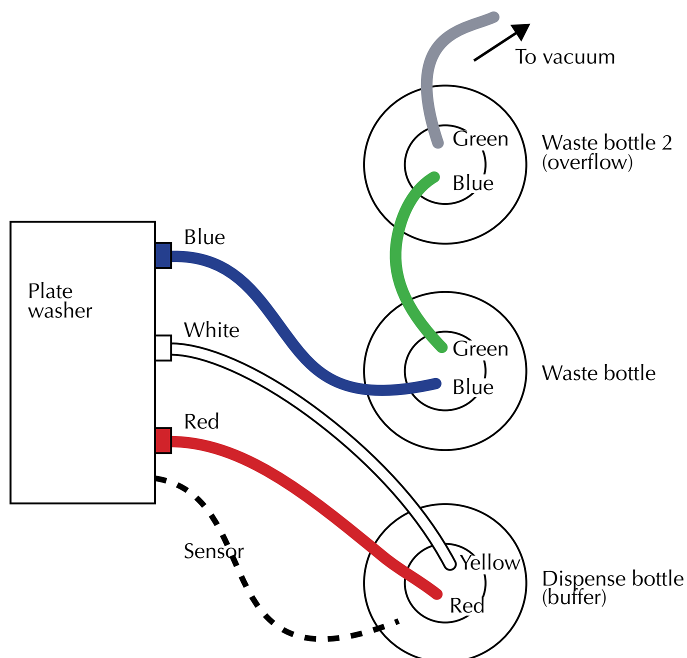

Figure 7-2: Connecting the bottles to the plate washer.

2) Connect the appropriate color-matched tubing to the rear of the plate washer and the vacuum (refer to Figure 7-3).

Note: *During calibration, the bottle assembly should be at the same level as the washer (i.e., on the same bench).*

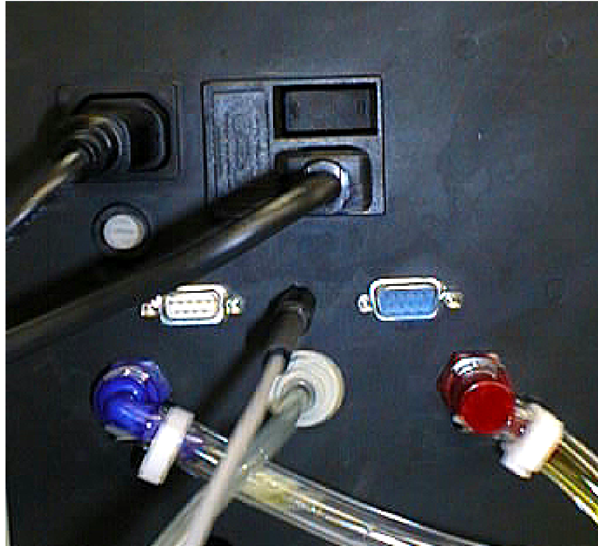

Figure 7-3: Rear panel connections.

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.

b) Vacuum Setup

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

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.*

7.2.5 Powering Up

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 *"Plate Washer Installation"* on page 139. 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.*

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 (see Figure 7-4).

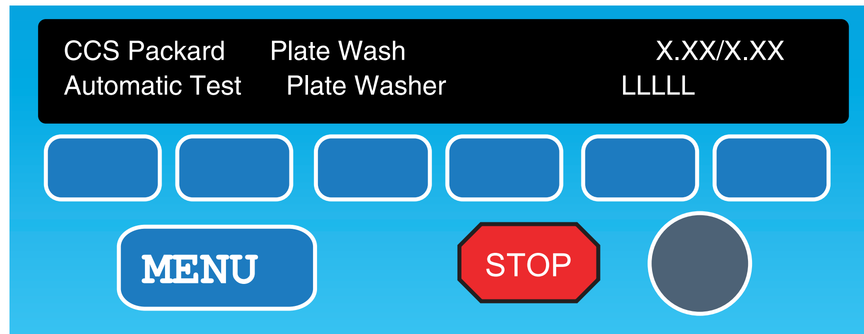

Figure 7-4: The **Start-Up** menu screen.

7.3 Using the Control Panel

7.3.1 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.

- **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, there may sometimes 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.

7.3.2 The Main Screen

All plate washer functions can be accessed from the Main screen (Figure 7-5). 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.

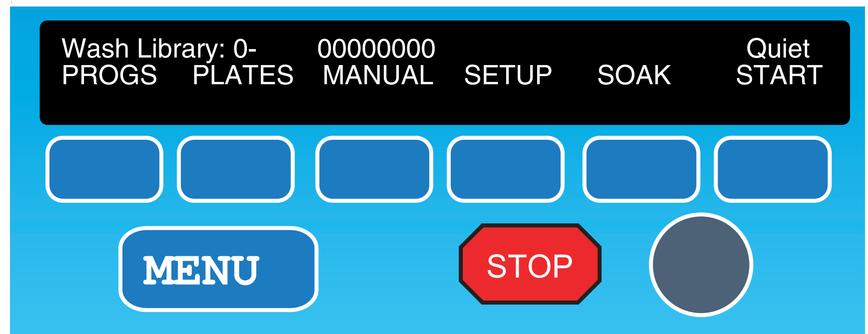

Figure 7-5: The **Main** screen.

a) 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:

- **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)

7.3.3 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 (refer to Figure 7-5).

To set the Quiet Mode time:

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.

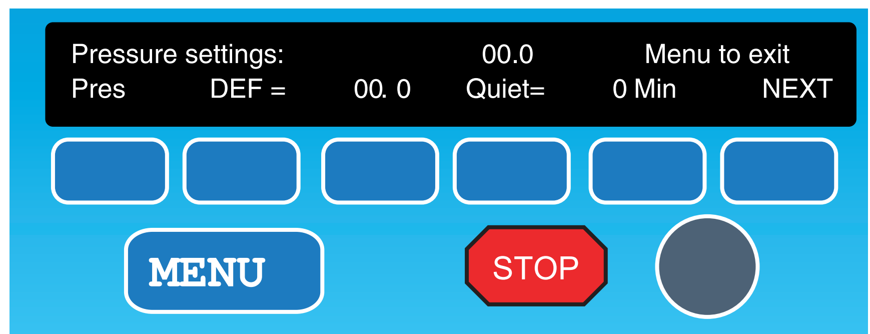

Figure 7-6: The **Quiet Mode** menu screen.

7.3.4 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.

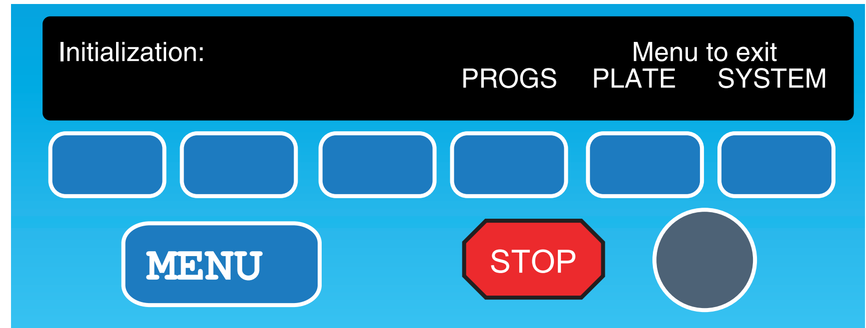

Figure 7-7: The **Initialization** menu screen.

7.3.5 The Option Menu Screen

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

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**

7.3.6 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:

1) Press the **Soak** softkey in the main screen to open the **Head Soak Setup** screen.

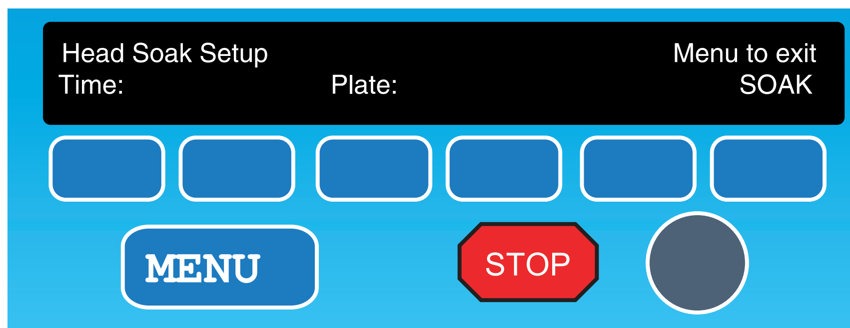

Figure 7-8: The **Head Soak Setup** screen.

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 (Figure 7-9).

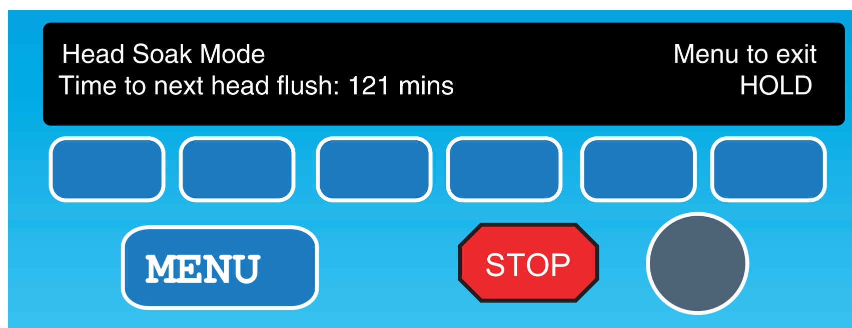

Figure 7-9: The **Head Soak Mode** screen.

5) Press the **Menu** softkey to terminate the soak function.

7.4 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:

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 (Figure 7-10).

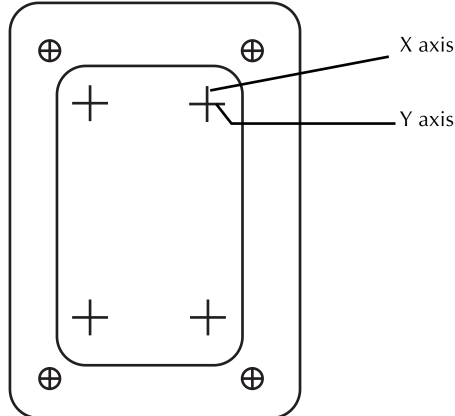

Figure 7-10: Setting the home position of the **X** and **Y** values with the cross hair.

6) Press the softkey beneath the **Y** value. Using the knob, position the wash head manifold until it touches the Y axis cross hair (refer to Figure 7-10). 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.*

7.5 Leveling the Wash Head Manifold

7.5.1 Leveling Procedure

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.

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**.

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.

- Counterclockwise: lowers
- Clockwise: raises

After each adjustment, verify that the plate carrier does not wobble. It is critical that all four leveling screws are in contact with the base unit.

7.6 Defining the Wash Pressure

7.6.1 Defining the Pressure Settings

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:

1) Starting from the main screen, press the **SETUP**′ **FLUIDS**′**NEXT** softkeys to display the **Pressure Settings** screen (Figure 7-11). The internal instrument pressure will be displayed on the top line.

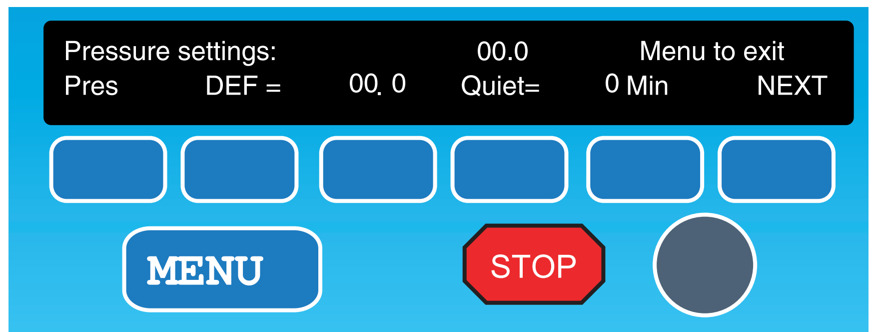

Figure 7-11: The **Pressure Settings** screen.

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).

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.*

7.7 Calibration and Initialization

7.7.1 Calibration

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

a) Preparing to Calibrate

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.

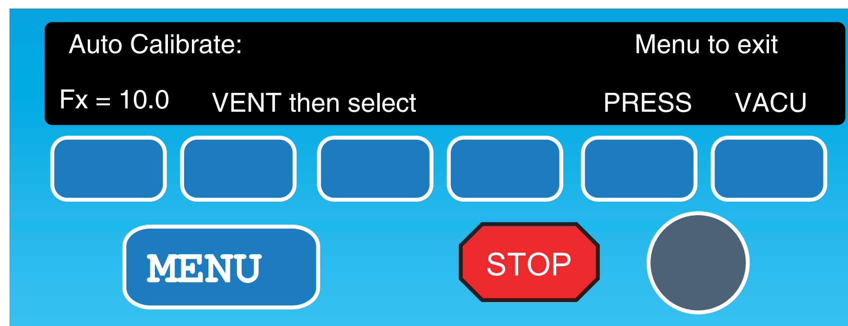

Figure 7-12: The **Calibration** screen.

3) Press the **VENT** softkey. The bleed valve will release the air and vacuum out of the system.

b) Calibrating the Vacuum

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 (Figure 7-13).

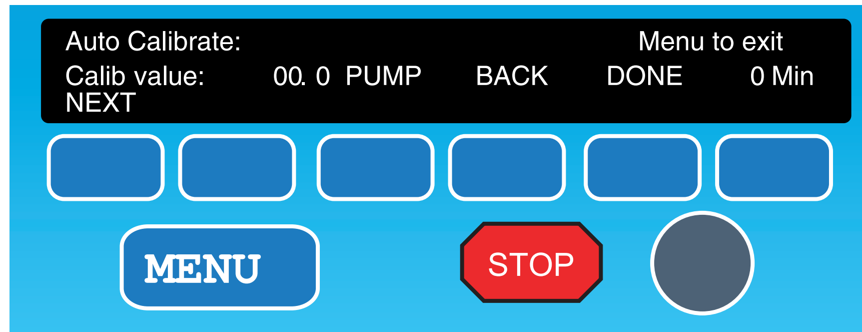

Figure 7-13: The pressure/vacuum **Auto Calibrate** screen.

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.

c) Calibrating the Pressure

1) Press the **PRES** softkey from the **Calibration** menu screen. The pressure/vacuum **Auto Calibrate** screen will appear (Figure 7-14).

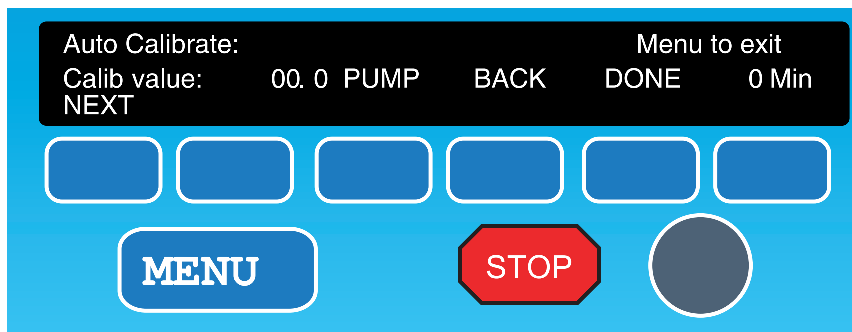

Figure 7-14: The pressure/vacuum **Auto Calibrate** menu screen.

2) Remove the air tubing connection in the rear panel of the plate washer.

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.

d) 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 Fx."*

7.8 Plate Definitions

7.8.1 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 in Figure 7-15. 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.

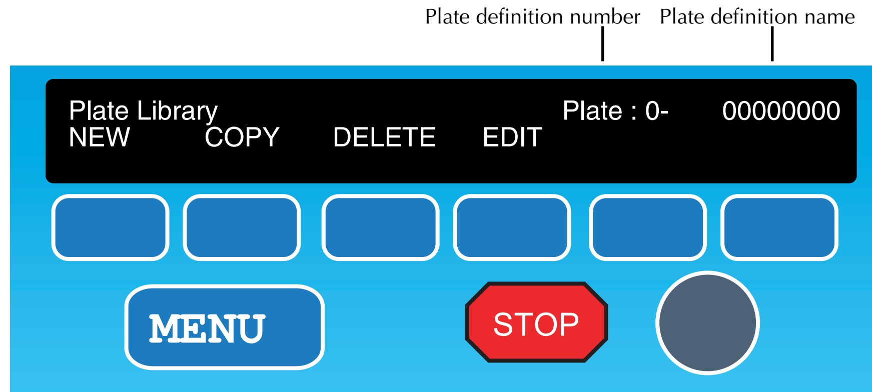

Figure 7-15: The **Plate Library** screen.

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 definitions 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.

7.8.2 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
$\mathbf{Y}_0$ 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

Table 7-3: Plate definition parameters and their accepted values.

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** positions 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.

a) Creating a New Plate Definition

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 (Figure 7-16).

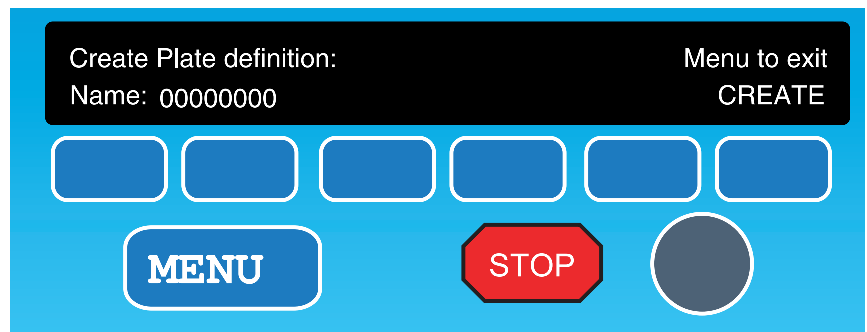

Figure 7-16: The **Create Plate definition** screen.

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.

4) After the name is entered, press the **CREATE** softkey to advance the screen to the **Edit Plate** menu screen (Figure 7-17).

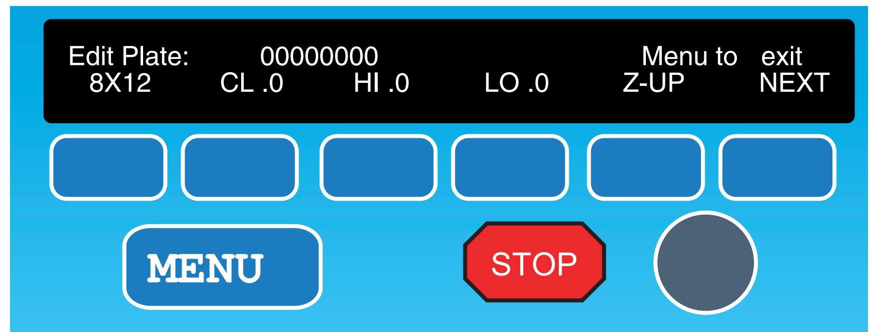

Figure 7-17: The **Edit Plate** screen.

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.

b) Defining a 96-well Plate

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 (Figure 7-18). Setting the XY coordinates prevents the wash probe manifold from being damaged.

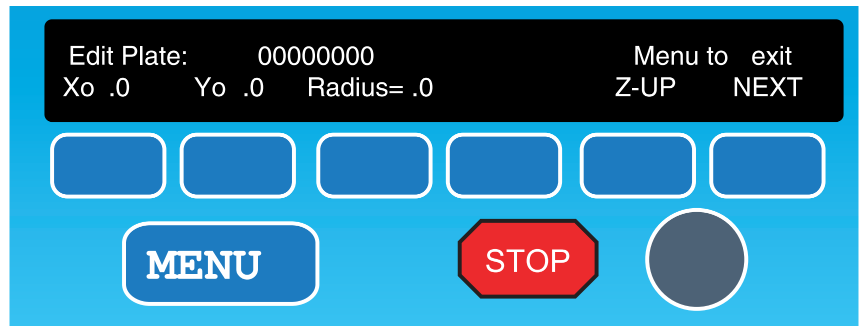

Figure 7-18: The **XY Coordinates** screen for 96-well plates.

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.

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

c) Defining a 384-well Plate

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: *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 (Figure 7-19). Setting the appropriate XY coordinates prevents the wash probe manifold from being damaged.

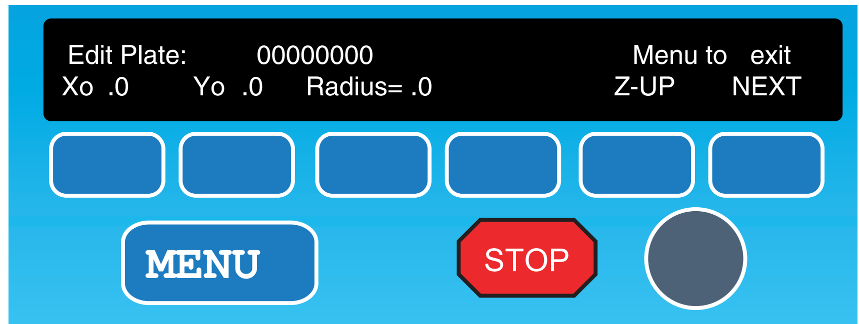

Figure 7-19: The **XY Coordinates** screen for 384-well plates.

3) Press the $\mathbf{X}_0$ softkey to set the X coordinate of the wash probe manifold for well A1 of the microplate.

4) Press the $\mathbf{Y}_0$ 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.

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.*

7.8.3 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.

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 section 7.8.2 for details on modifying plate definitions).

7.8.4 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.

7.8.5 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 section 7.8.2 for details on modifying plate definitions).

7.9 Wash Programs

7.9.1 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 section 7.8 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 nine 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

Table 7-4: Wash program parameters and their acceptable values.

7.9.2 Creating a New Wash Program

1) Press the **PROG** softkey in the main screen to open the **Program Library** screen (Figure 7-20). 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.

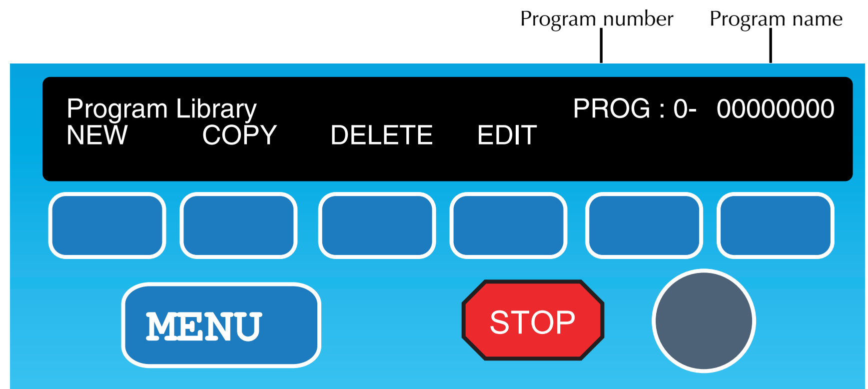

Figure 7-20: The **Program Library** screen.

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.

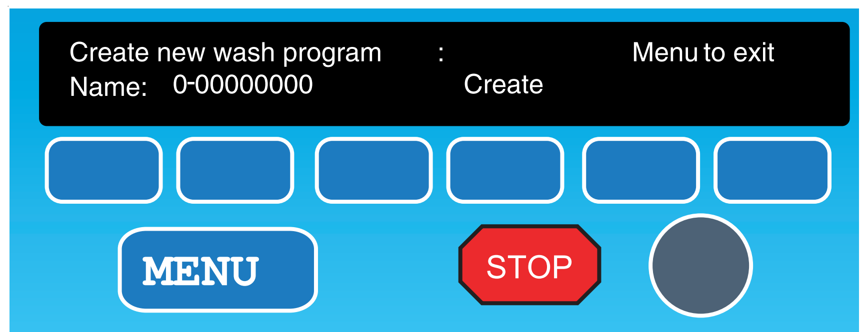

Figure 7-21: The **Create New Wash Program** screen.

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 (Figure 7-22).

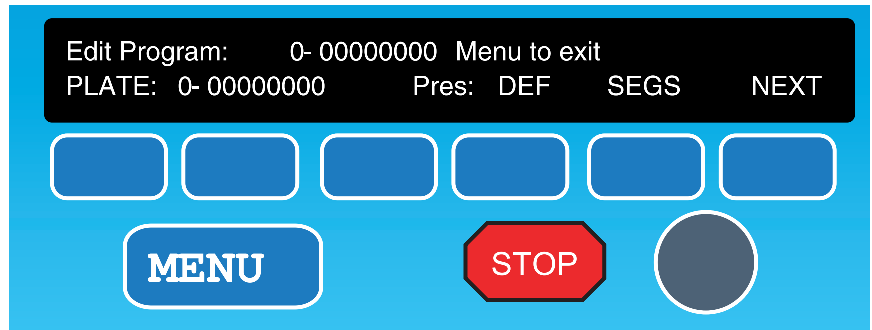

Figure 7-22: The **Edit Program** screen.

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 (Figure 7-23).

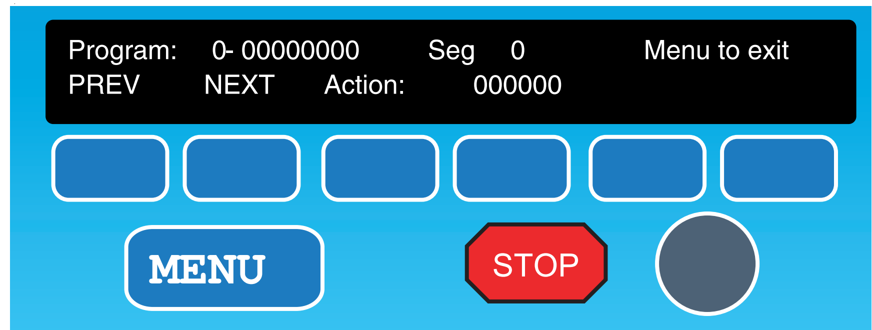

Figure 7-23: The segment programming screen.

Note: *The* **NEXT** *softkey is not used with the FLIPR^{384} system integration.*

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 times
MOVE	Probe height	0.0–50.0 mm
END	NA	NA

Table 7-5: Parameters and values for washer actions.

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.*

7.9.3 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.

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 7.9.2 for details on modifying wash programs).

7.9.4 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.

7.9.5 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 (see section 7.9.2 for details on modifying wash programs).

7.10 Running Wash Programs

7.10.1 Preparing to Run a Wash Program

a) Plate Washer Checklist

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

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.

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 inlet
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

Table 7-6: External connections to the FLIPR^{384} plate washer.

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.

b) Priming the Plate Washer

1) Press the **SETUP** softkey in the main screen.

2) Press the **FLUIDS** softkey to advance to the **Fluid Operations** menu screen (Figure 7-24).

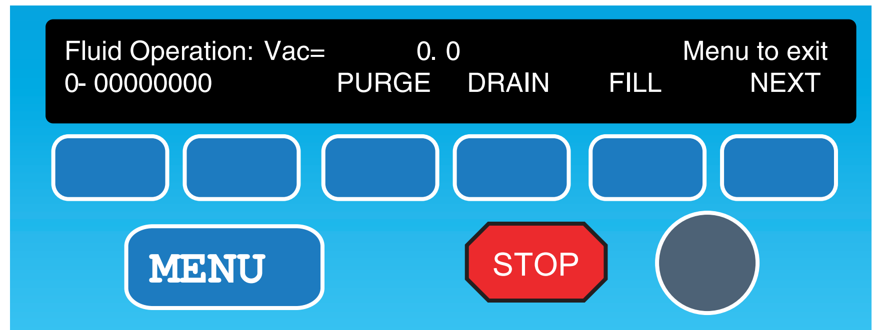

Figure 7-24: The **Fluid Operation** screen.

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.

7.10.2 Running a Wash Program

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. Figure 7-25 illustrates the designations of the individual quadrants.

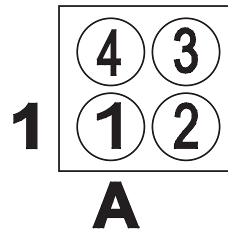

Figure 7-25: Quadrant assignment in a 384-well plate.

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.

7.10.3 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:

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.

7.11 Manual Operation

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

1) Press the **MANUAL** softkey in the main screen to enter the **Manual Operation** screen.

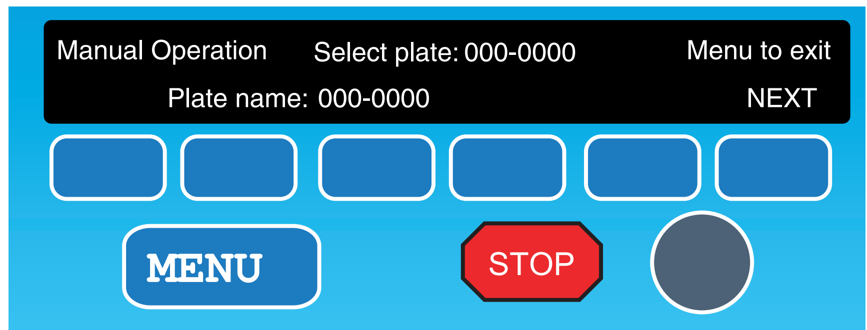

Figure 7-26: The **Manual Operation** screen.

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.

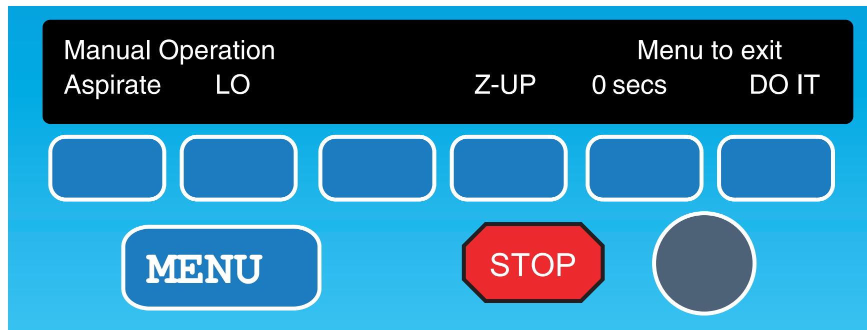

Figure 7-27: The second **Manual Operation** screen.

5) Select the manual operation to be performed. Refer to Table for the programmable options for each manual operation.

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**

Table 7-7: Programmable options for manual operation of the plate washer.

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.*

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

7.12 Setup Parameters

7.12.1 Determining the Residual Volume

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

1) Create a plate definition for the cell plate, including the **X**, **Y**, **Clearance** (**CL**) and **HI** positions by inspection (see section 7.8.2, *"Creating Plate Definitions"* on page 155 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,

$$\left(\frac{\text{change in cell plate weight}}{\text{buffer density} \times 384}\right) \times 1000$$

   For 96-well plates,

$$\left(\frac{\text{change in cell plate weight}}{\text{buffer density} \times 96}\right) \times 1000$$

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.)

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.

7.12.2 Determining the Maximum Washing Volume

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.

7.12.3 Wash Program Parameters

Initially, we recommended a sequential dispense and aspirate wash program. The following wash parameters are valid for both 384- and 96-well plates (see section 7.9.2, *"Creating a New Wash Program"* on page 163 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		

Table 7-8: Wash program segments and their definable parameters.

7.12.4 Experimental Setup Parameters

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.

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.

7.12.5 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.

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.

7.13 Maintenance

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

7.13.1 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.

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).

7.13.2 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
Leveling the wash probe manifold			x
Blocked probes			x

Table 7-9: Plate washer maintenance schedule.

7.13.3 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.

7.14 System Decontamination Procedure

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.

7.14.1 System Decontamination Procedure

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.

7.14.2 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.*

7.14.3 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.*

7.15 Shipping the Plate Washer

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:

1) 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.

2) Instruments returned to the manufacturer for repair require a Certificate of Decontamination. Decontaminate the instrument as detailed in section 7.14, starting on page 179. Photocopy the Certificate of Decontamination located in Appendix D and fill it out completely. Include the certificate with all appropriate documentation.

7.15.1 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.

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.

7.15.2 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.

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

7.16 Trouble-shooting and Validation

7.16.1 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.

Problem	Probable Cause
Excessive residual fluid after aspiration (evenly distributed)	Lack of vacuum (15 in. Hg @ 2CFM) or the wash manifold is too high for a particular plate
Excessive residual fluid after aspiration (unevenly distributed)	Wash probe manifold is not level
Fluid being removed during soak operation	Leaking aspiration valves or dirty vent filter
Uneven dispensing	Lack of fluid pressure or large air bubbles in the wash dispense head or the position of the dispense probe
≥1 wells not filling	Clogged probe
Dripping wash head	If sufficient vacuum is being supplied, possible air leak through the manifold or valves

Table 7-10: Troubleshooting symptoms and their possible causes.

Also, see section 7.12 , *"Setup Parameters"* on page 172 for additional information on configuring the plate washer correctly.

7.16.2 Validation

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.

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**.
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

Chapter 8
Maintenance

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8.1 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 that can affect the coefficient of variation.*

8.1.1 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 Overview"* for details).

8.1.2 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 Overview"* for details).

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

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 Correction Protocol,"* for details).

4) Print the results and keep them in a "Maintenance" folder by the instrument to track the instrument performance over time.

8.2 Changing the Plate Mask or 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). To change the mask or ledge:

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 (Figure 8-1).

Important: *The plate ledge must be installed with its smooth side against the bottom of the stage.*

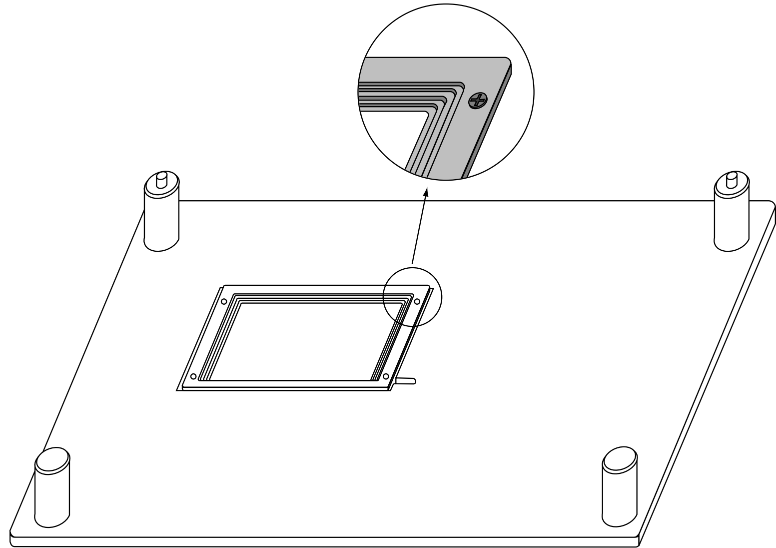

Figure 8-1: The underside of the stage when the ledge is installed. Note that the concentric “steps” on the ledge are installed facing away from the underside of the stage.

8.3 Laser Mirror Alignment

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.

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 Correction Protocol,"* for details).

Warning: *Never touch both silver knobs at the same time.*

8.4 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 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!

8.5 Air Flow

Maintain a reliable air source at 90–100 psi. The pipette tips' sealing system and the heated stage lids use air and will not function properly unless the air pressure is sufficient. Insufficient air pressure will cause the message "Air supply is off!" to appear in the Interlock section of the software's FLIPR Status Bar, and the experiment will be halted.

8.6 Camera Alignment

The FLIPR system computes the fluorescence signal differently, depending on the type of plate (384- or 96-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.

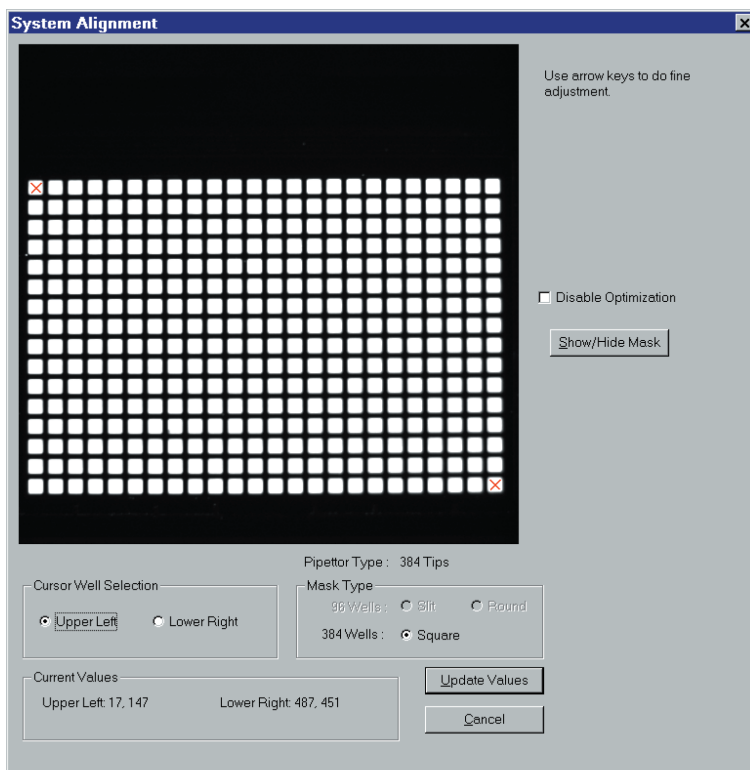

Figure 8-2: The **System Alignment** dialog box.

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.

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

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

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

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.

8.7 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.*

8.8 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 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.

Chapter 9
Troubleshooting

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9.1 Introduction

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.

9.2 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.

Symptom or Error Message	Possible Causes	Solutions
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.
"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.

9.3 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.

9.4 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.
Wells are cut off in plate viewer.	Change in the alignment settings.	Perform Optical Correction Protocol (see Appendix E, "*Optical Correction 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.

Symptom or Error Message	Possible Causes	Solutions
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 Correction Protocol"*).
	Stage was changed or moved.	Perform Optical Correction Protocol (see Appendix E, *"Optical Correction 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.

9.5 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).
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.

Symptom or Error Message	Possible Causes	Solutions
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.

9.6 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.

Symptom or Error Message	Possible Causes	Solutions
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.

9.7 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 Section 5.8, *"Optimizing an Assay"* on page 117.
	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."*

Symptom or Error Message	Possible Causes	Solutions
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 Section 5.8 *"Optimizing an Assay"* on page 117.
	Tip problem.	Contact Technical Support.

9.8 General

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.

9.9 Stacker

Symptom or Error Message	Possible Causes	Solutions
Stacker stage does not move plate into position.	Various possible causes.	Contact Technical Support.
Stacker moves cell plate too quickly or slowly and the wells are splashed	Stacker stage requires calibration.	Contact Technical Support.
Bar code reader mis-reads the bar code and filename reads “Badbarcode.”	Misplacement of bar code or incor-rect type of barcode.	Bar code must be on long side of plate. Place label as low as possible. Use labels with numbers on top.

9.10 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 artifi-cial spikes at random times.	CCD camera or controller problem.	Contact Technical Support.

Appendix A

Consumables Used With the FLIPR System

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	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
	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	356663
Black wall plates, clear bottom, tissue culture treated, sterile, 384-well, *collagen coated*	Becton Dickinson	800-343-2035	356667

384-well Clear Plates

384-well Clear Plates	Suggested Supplier	Phone Number	Item #
Clear plate for compounds, 384-well, polypropylene, with lid	Greiner (distributed by E& K)	408-378-2013	781201

Pipetting Accessories

Item	Suggested Supplier	Phone Number	Item #
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
FLIPR pipette tips, black, non-sterile, 96-well, 250 µL	Molecular Devices	800-635-5577 408-747-1700	9000-0549
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 tips for FLIPR384 96-well format: Autotips, black, non-sterile, 96-well, 200 µL	Robbins Scientific	800-752-8585 408-734-8500	1043-24-5
For FLIPR384 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 (for pipettor calibration), 96-well, 250 µL	Molecular Devices	800-635-5577 408-747-1700	9000-0548
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
FLIPR pipette tips, clear, 384-well, type A (fits on 384 head type A)	Molecular Devices	800-635-5577 408-747-1700	9000-0257
FLIPR pipette tips, clear, 384-well, type B (fits on 384 head type B)	Molecular Devices	800-635-5577 408-747-1700	9000-0512

Calcium Flux Assay Consumables

Item	Suggested Supplier	Phone Number	Item #
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
Calcium Assay Reagent Kit	Molecular Devices	800-635-5577 408-747-1700	R-8033
DMSO, low water content	Sigma	800-325-3010	D2650
Aspirator manifold 12 pin	Wheaton Science	800-225-1437	851388
Aspirator manifold 8 pin	Wheaton Science	800-225-1437	851381

Membrane Potential Assay Consumables

Item	Suggested Supplier	Phone Number	Item #
Hank's Balanced Salt Solution (10X stock)	Gibco	800-828-6686	14065-056
HEPES buffer solution 1X	Irvine Scientific	800-437-5706	9319
Membrane Potential Assay Reagent Kit	Molecular Devices	800-635-5577 408-747-1700	R-8034
FLIPR Emission Filter (540–590 nm band pass)	Molecular Devices	800-635-5577 408-747-1700	0310-4027
DMSO, low water content	Sigma	800-325-3010	D2650
UTP, Na salt (receptor-mediated positive control)	Sigma	800-325-3010	U-6625
Ionomycin (positive control)	CalBiochem	800-854-3417	407950
Aspirator manifold 12 pin	Wheaton Science	800-225-1437	851388
Aspirator manifold 8 pin	Wheaton Science	800-225-1437	851381

Important equipment not supplied with your FLIPR System:

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

Other items needed and used in a cell culture facility:

Item	Suggested Supplier	Phone Number
Multidrop 96/384	Labsystems	800-522-7763 508-541-0444
16-channel pipettor	Labsystems	800-522-7763 508-541-0444
12-channel Impact 2 pipettor (15–850 µL)	Matrix	800-345-0206
1250 µL pipet tips for Matrix Impact	Matrix	800-345-0206
Manual12-channel pipettor 50–200 µL	Brinkman	
Sterile pipette tips 200 µL	E+K Scientific	408-378-2013

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

Appendix B

Improving Confluence and Adherence of Weakly-adherent Cells

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.

B.1 Poly D-lysine Coating

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.

B.2 Laminin Coating

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.

B.3 Collagen Coating

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) Aspirate the collagen with a Pasteur pipet.

4) The next day, rinse once with sterile PBS 1X to neutralize the pH.

Appendix C
FLIPR Systems Data Processing Algorithms

Consider the results from a hypothetical 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, 6 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 9 wells are considered.)

Table C-1 presents the results of the experiment without **Spatial Uniformity Correction**, **Negative Control Correction**, **Positive Control Scaling**, or **Subtract Bias Value**.

Note: *In the FLIPR Control software,* **Subtract Bias Value** *is turned on by default.*

Sampl e	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
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

Table C-1: Unprocessed FLIPR data

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.

C.1 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.

1) The correction factor is derived by calculating the mean fluorescent counts of all wells at Sample 1 (Table C-2).

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

Table C-2: Spatial uniformity correction

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.*

2) 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.) Table C-2 also presents the correction factor for wells A1–A9.

3) 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 Table C-3. 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
6	30	9856	8925	8930	32700	33950	29580	44500	47940	51500

Table C-3: FLIPR data after spatial uniformity correction has been applied

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.

C.2 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.

1) 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

Table C-4: Negative control correction factor

2) The mean of Sample 1 is divided by the mean of each of the samples to give the sample-specific correction factor.

3) 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

Table C-5: FLIPR data after negative control correction has been applied

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.*

C.3 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 for factors such as cell density, cell response, laser power, or exposure time. This algorithm also makes EC_{50} comparisons easier.

1) 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

Table C-6: Positive control scaling calculation

2) The difference in fluorescence counts between Sample 1 and all of the samples is calculated.

3) The greatest difference is determined.

4) 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.

5) All samples are multiplied by the correction factor 0.0024 (Table C-7).

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

Table C-7: FLIPR data after positive control scaling has been applied

Note that all wells, at samples 1–3, range from 20–23%. The positive control wells at Sample 4 range from 114 to 130% while the negative control wells remain at around 20% throughout the experiment.

C.4 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.

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.

1) Sample 1 (or any sample chosen by the operator) from each well is subtracted from the samples from the same well (Table C-8).

Sample	Time	Well								
		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

Table C-8: FLIPR data after bias has been subtracted

The results in Table C-8 only 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.

Appendix D

Calibrating the Plate Washer Fx

Note: *The Fx factor is set in the factory, and does not need to be routinely reset by the user.*

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 (Figure D-1).

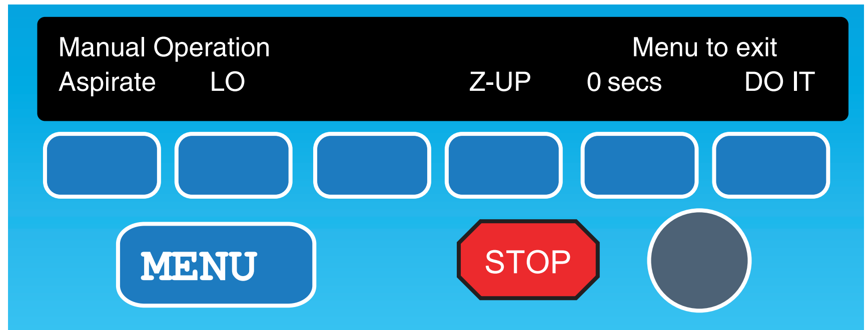


Figure D-1: The second **Manual Operation** screen.

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 7.8.2, *"Creating Plate Definitions"* on page 155 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.

When the calculated volume approximates the dispensed volume, the calibration of Fx is complete.

Appendix E
Optical Correction Protocol

E.1 Preliminary Setup

1) Power on the laser subsystem and allow it to stabilize for 0.5 to 1 hour.

2) Turn on the FLIPR system and the camera controller. Be sure the controller temperature has stabilized before proceeding.

3) Have 96- or 384-well yellow plate available (as appropriate to your assay.)

E.2 Delete the Existing Calibrate.*** File

Before a new calibration file can be generated, the old or current file must be deleted:

1) From the Windows **Start** menu, choose **Start fiPrograms fi Windows NT Explorer**.

2) Use the Explorer to open the **C:** drive, then select and open the **FLIPR** folder, then select and open the **Bin** folder within it.

3) Locate and select the file named **Calibrate.384** or **Calibrate.96** (The file name will depend on which type of cell plate is being calibrated.)

4) Click the right mouse button to display a pop-up menu of options. Select **Delete**. When prompted to confirm the deletion, select **Yes**.

5) Minimize the **NT Explorer** window.

E.3 Creating the First Set of Calibration Data

Before creating the new calibration file, check that the appropriate mask and yellow plate are being used. Also check that the yellow plate is free of fingerprints, dust, dirt, and scratches.

1) Double click on the FLIPR icon to open FLIPR Control Software. A message will appear, indicating there is no

calibration file for the plate format deleted in previous steps. Click the **Okay** button.

2) Select **Experiment** from the **Setup** menu to open the Experiment Setup dialog (Figure E-1).

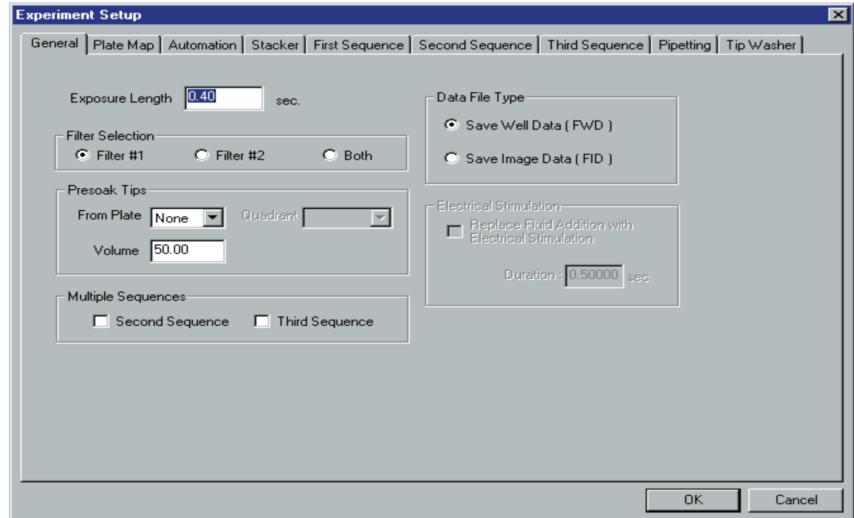
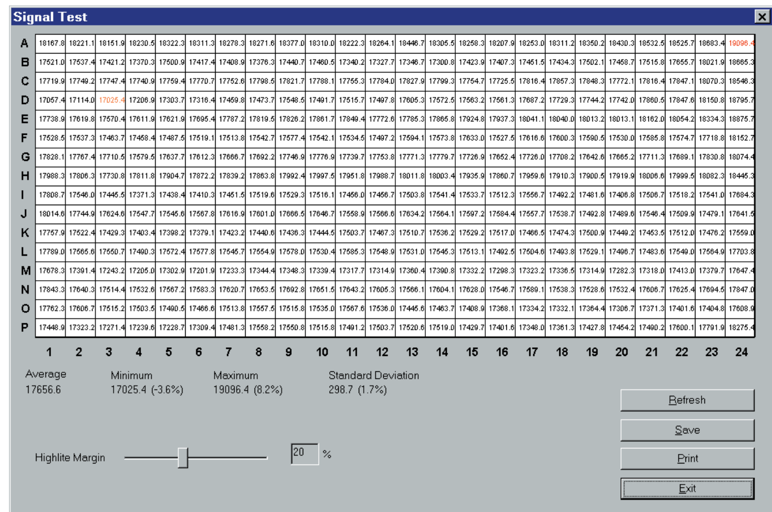


Figure E-1: The **Experiment Setup** dialog box.

3) Change the exposure time to 0.05 seconds, then click **OK**.

4) Place the yellow plate on the stage in the cell plate position (or use the stacker to do this).

5) Click the Light Bulb icon in the FLIPR tool bar to execute the signal test. (This can also be accomplished by selecting **Signal Test** from the **Run** menu.) The results will be displayed in the **Signal Test** dialog box (Figure E-2).



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	18167.8	18221.1	18151.9	18230.5	18322.3	18311.3	18278.3	18271.6	18377.0	18310.0	18222.3	18264.1	18446.7	18305.5	18258.3	18207.9	18253.0	18311.2	18350.2	18430.3	18532.5	18525.7	18683.4	19096.4
B	17521.0	17537.4	17421.2	17370.3	17500.9	17417.4	17408.9	17376.3	17440.7	17460.5	17340.2	17327.7	17346.7	17300.8	17423.9	17407.3	17451.5	17434.3	17502.1	17458.7	17515.8	17655.7	18021.9	18665.3
C	17719.9	17749.2	17747.4	17740.9	17759.4	17770.7	17752.6	17798.5	17821.7	17788.1	17755.3	17784.0	17827.9	17799.3	17754.7	17725.5	17816.4	17857.3	17848.3	17772.1	17816.4	17847.1	18070.3	18546.3
D	17057.4	17114.0	17025.4	17206.9	17303.7	17316.4	17459.8	17473.7	17548.5	17491.7	17515.7	17497.8	17605.3	17572.5	17563.2	17561.3	17687.2	17729.3	17744.2	17742.0	17860.5	17847.6	18150.8	18795.7
E	17738.9	17619.8	17570.4	17611.9	17621.9	17695.4	17787.2	17819.5	17826.2	17861.7	17849.4	17772.6	17785.3	17865.8	17924.8	17937.3	18041.1	18040.0	18013.2	18013.1	18162.0	18054.2	18334.3	18875.7
F	17528.5	17537.3	17463.7	17458.4	17487.5	17519.1	17513.8	17542.7	17577.4	17542.1	17534.5	17497.2	17594.1	17573.8	17633.0	17527.5	17616.6	17600.3	17590.5	17530.0	17585.8	17574.7	17718.8	18152.7
G	17828.1	17767.4	17710.5	17579.5	17637.7	17612.3	17666.7	17692.2	17746.9	17776.9	17739.7	17753.8	17771.3	17779.7	17726.9	17652.4	17726.0	17708.2	17642.6	17665.2	17711.3	17689.1	17830.8	18074.4
H	17988.3	17806.3	17730.8	17811.8	17904.7	17872.2	17839.2	17863.8	17992.4	17997.5	17951.8	17988.7	18011.8	18003.4	17935.9	17860.7	17959.6	17910.3	17900.5	17919.9	18006.6	17999.5	18082.3	18445.3
I	17808.7	17546.0	17445.5	17371.3	17438.4	17410.3	17451.5	17519.6	17529.3	17516.1	17456.0	17456.7	17503.8	17541.4	17533.7	17512.3	17556.7	17492.2	17481.6	17406.8	17506.7	17518.2	17541.0	17684.3
J	18014.6	17744.9	17624.6	17547.7	17545.6	17567.8	17616.9	17601.0	17666.5	17646.7	17558.9	17566.6	17634.2	17564.1	17597.2	17584.4	17557.7	17538.7	17492.8	17489.6	17546.4	17509.9	17479.1	17641.5
K	17757.9	17522.4	17429.3	17403.4	17398.2	17379.1	17423.2	17440.6	17436.3	17444.5	17503.7	17467.3	17510.7	17536.2	17529.2	17517.0	17466.5	17474.3	17500.9	17449.2	17453.5	17512.0	17476.2	17559.0
L	17789.0	17565.6	17550.7	17490.3	17572.4	17577.8	17545.7	17554.9	17578.0	17530.4	17585.3	17548.9	17531.0	17545.3	17513.1	17492.5	17504.6	17493.8	17529.1	17496.7	17483.6	17549.0	17564.9	17703.8
M	17678.3	17391.4	17243.2	17205.0	17302.9	17201.9	17233.3	17344.4	17348.3	17339.4	17317.7	17314.9	17360.4	17390.8	17332.2	17298.3	17323.2	17336.5	17314.9	17282.3	17318.0	17413.0	17379.7	17647.4
N	17843.3	17640.3	17514.4	17532.6	17567.2	17583.3	17620.7	17653.5	17692.8	17651.5	17643.2	17605.3	17566.1	17604.1	17628.0	17546.7	17589.1	17538.3	17528.6	17532.4	17606.7	17625.4	17694.5	17847.0
O	17762.3	17606.7	17515.2	17503.5	17490.5	17466.6	17513.8	17557.5	17515.8	17535.0	17567.6	17536.0	17445.6	17463.7	17408.9	17368.1	17334.2	17332.1	17364.4	17306.7	17371.3	17401.6	17404.8	17608.9
P	17448.9	17323.2	17271.4	17239.6	17228.7	17309.4	17481.3	17558.2	17550.8	17515.8	17491.2	17503.7	17520.6	17519.0	17429.7	17401.6	17348.0	17361.3	17427.8	17454.2	17490.2	17600.1	17791.9	18275.4

Figure E-2: The **Signal Test** dialog box.

6) Verify that the minimum ≥ -18.0%, and the maximum ≤ 18.0%, and the standard deviation is ≤ 8.0%.

7) The test results need to be saved into the **Bin** folder in the **FLIPR** folder. Click the **Save** button, then use the **Save** dialog box to select **C:** drive → **FLIPR** directory → **Bin** folder (Figure E-3).

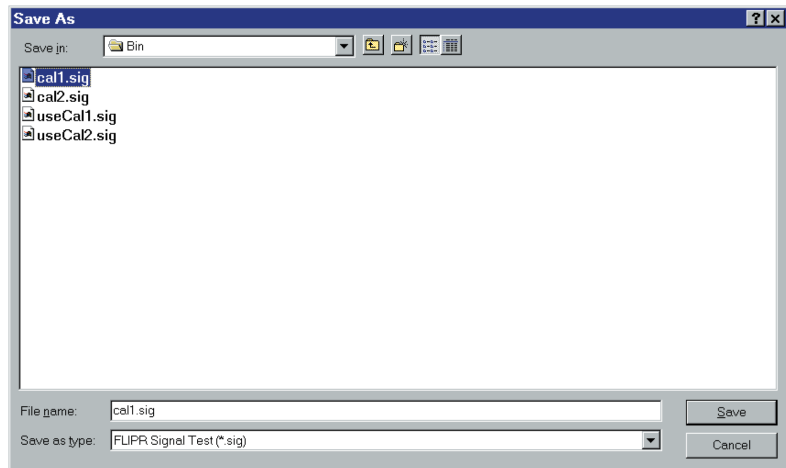


Figure E-3: The **Save As** dialog box.

Select **Cal1.sig** from the list of file names. If the file is not present, check that you are in the **FLIPR** directory → **Bin** folder.

An alert dialog will appear, warning you that the existing file called **Cal1.sig** will be overwritten. Click **OK** in the alert dialog to overwrite the old file, then click **OK** to save the file.

8) Exit the **Signal Test** dialog box.

E.4 Creating the Second Set of Calibration Data

1) Open the FLIPR door by selecting the door icon on the FLIPR toolbar, then rotate the yellow plate 180°. If using the stacker, rotate the plate 180°, then replace the plate in the cassette.

2) Repeat the steps in section Figure E.3 to execute the signal test, saving the new signal test results as **Cal2.sig**, overwriting the existing file of the same name.

3) Close the **Signal Test** dialog box and exit the FLIPR Control Software.

E.5 Specifying the New Calibration Files

1) Maximize the **NT Explorer** window, then left-double click on **384Calibrator.exe** or **96Calibrator.exe** (the file name will depend on the type of cell plate being calibrated). A new file window will be created. Close it by clicking on the 'X' in its upper right corner.

2) Once this file is closed, there will be a new file named **Cal.96** or **Cal.384** in the **Bin** folder. The file must be renamed "**Calibrate.96**" or "**Calibrate.384**".

3) Select the file, and use the right mouse button to display a pop-up menu of options, then select **Rename**.

4) Type in the new file name: **Calibrate.96** or **Calibrate.384**.

Note: *The file name is case-sensitive, and must be entered exactly as shown.*

5) Exit **NT Explorer**.

6) Repeat the steps in section Figure E.3 of this protocol to run a signal test. If the warning that no calibration will be applied re-appears, check that the name of the file saved in Step 4 above was correctly entered as "**Calibrate.96**" or "**Calibrate.384**".

7) Verify that the resultant standard deviation ≤ 3.5%.

Appendix F
Decontamination Certificate

Circle the appropriate statement and complete both pages of the form as necessary.

1) The instrument or parts that are being returned for rework were not used for any application involving blood or other potentially infectious material.

2) The instrument or parts that are being returned for rework have been decontaminated.

3) 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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