Q-Guide:

Assay Design and Data Acquisition

This Q-Guide is a companion piece to the video tutorial series for ForeCyt 3.0. ForeCyt is the software package that operates IntelliCyt's screening systems including the HTFC® and iQue™ Screener.

In This Guide

Overview

This section details how to design experiments and collect data. Information regarding the contents of the plate, the manner in which the plate or multiple plates were sampled and how data were collected and analyzed are included in the experiment.

Creating a New Experiment

1. Double-click the **ForeCyt icon**  to start the application.
2. Select the **New Experiment** button, or choose **File>New Experiment.**
   The New Experiment window appears.
3. Type a unique name for the experiment in the **Experiment Name** field. If an experiment has the same name as an already-existing experiment, the system will append a "_1" to the name of the new experiment.

> **NOTE**
>
> The name may contain letters, numbers, and certain symbols. The following symbols are NOT allowed in experiment names: < > : " / \ | ? *

4. Select the drop-down arrow next to **Plate Type** to choose a plate model for the experiment format (**Test Tube, 96 Well** or **384 Well**).
5. Select a folder in which to store your experiment. Choose an existing project folder or create a **New Folder** for the experiment. Create new, delete, or rename folders using the buttons below the folder tree pane.

> **NOTE**
>
> The folders are stored within the system's database, and will not appear in the Windows file system. See Q-Guide: Data Management for more information about Data Management using the folder tree.

6. Select **Blank Experiment** to create a new experiment with no pre-configured settings or analysis features. Select **Use Template** to import the design and protocol from an existing template.
7. Select **Save** to create the new experiment.

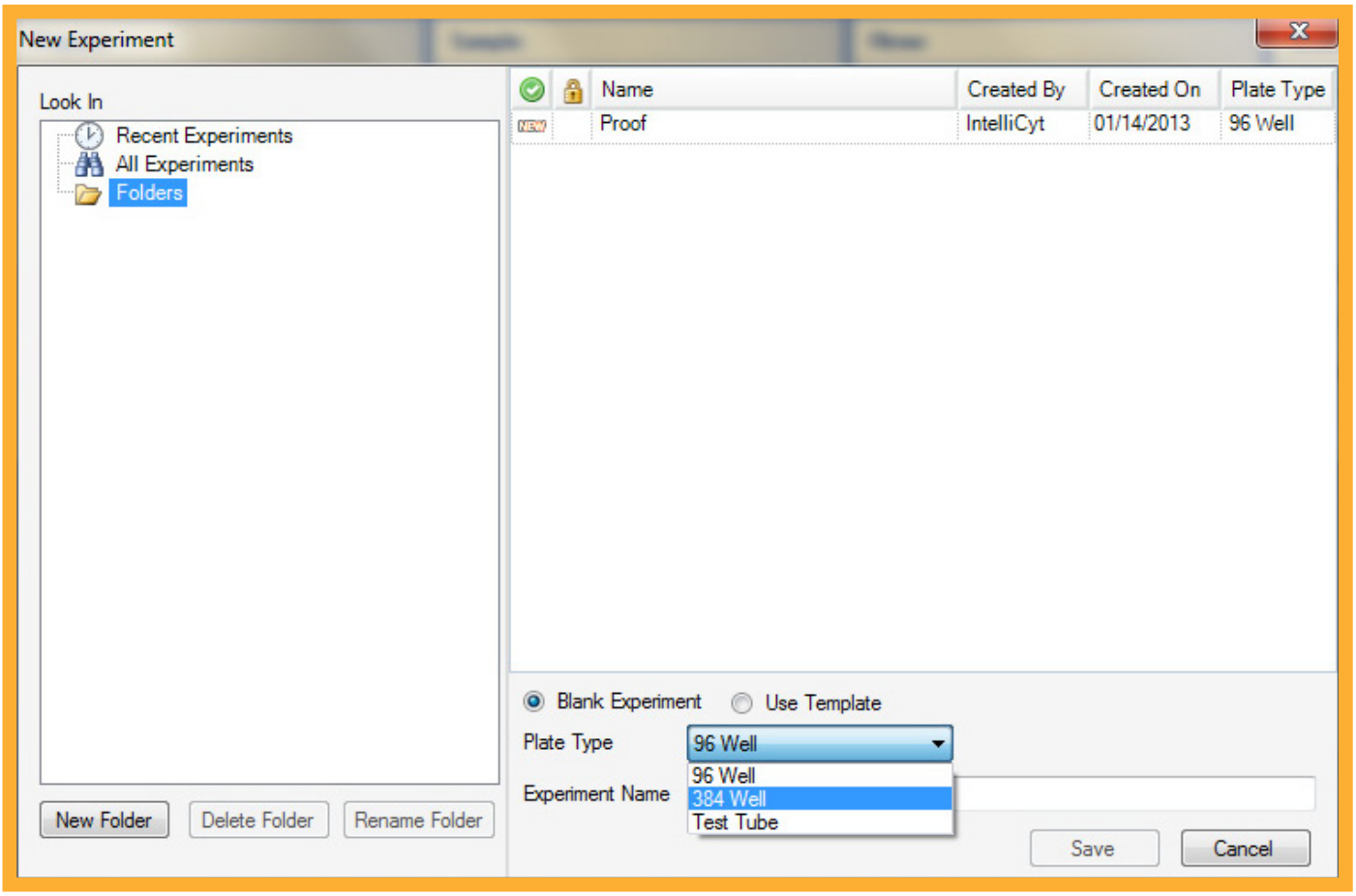

Figure 1. The New Experiment Pane.

Visit the link below to watch a video tutorial on Templates.
http://www.intellicyt.com/training/templates

Design Tab

Experiment Pane

Name: Displays the experiment name designated in the previous section. The experiment name cannot be edited from this field. To edit the experiment name, use **File>Manage Experiments**.

Notes: Text entry field where additional information can be typed, and will be kept with the experiment

Status: Select the **Lock** button to make the experiment read-only. The phrase "[read-only]" will appear as part of the experiment title of a read-only experiment. Modifications made to a read-only experiment may only be saved as another experiment (Save As).

NOTE

Once the experiment is locked, the Lock button will change to read Unlock. Select this button if changes need to be made to the experiment.

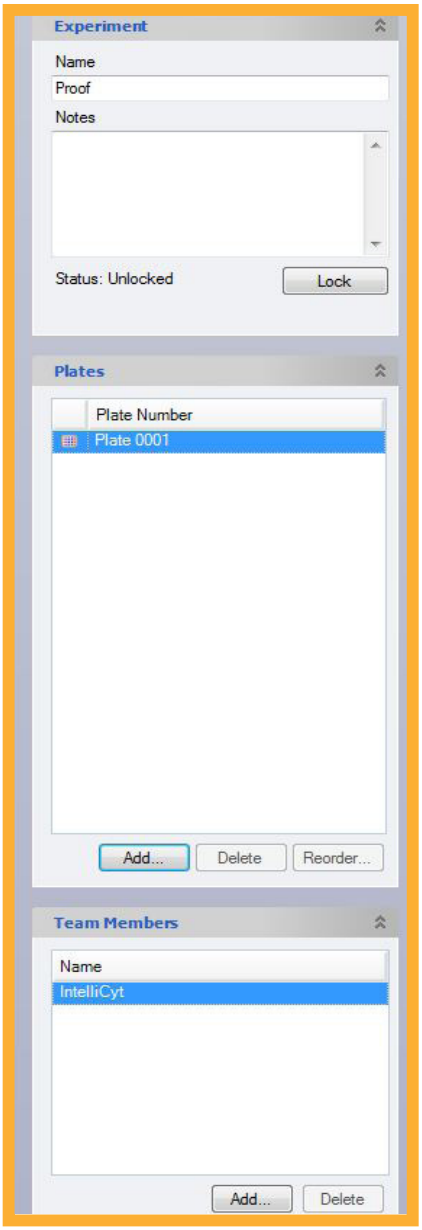

Figure 2. Experiment Pane

Plates Pane

Add multiple plates to the experiment using the Plates pane of the Experiment Setup tab. Use the **Add**, **Delete**, and **Reorder** buttons to manage plates in multiple-plate experiments.

1. Choose **Add** to add one or more new plates.
2. Select **OK** to add the plates to the list. Switch the plate that currently displays by highlighting its name in the Plates pane list.
3. Reorder the list of plates (optional). Select the **Reorder** button. Select the plate and use the up/down arrows to move it to the desired location within the list.

Team Members Pane

Scroll down to the Team Members pane to edit.

The current user name will appear under **Name** in the **Team Members** pane. An experiment with a single user means that the experiment may only be edited, analyzed, and saved by that specific user. Adding additional users allows multiple users to make changes or acquire data on a single experiment. If a user who is not listed as a team member attempts to open the experiment, they will launch a [read-only] copy of the experiment.

Additional members may be added who are registered as users in the IntelliCyt Manager. See Q-Guide: IntelliCyt Manager for more information about configuring users.

To Add Additional Users to the Team:

1. Select **Add** to bring up the Add Team Members window.
2. Highlight the desired name(s) and select **Add**.
3. Team members can be deleted by highlighting the member's user name and selecting **Delete**.

> **NOTE**
>
> You cannot delete yourself from the Team Member List.

Identify the Wells to Sample

1. Within the plate layout workspace, click and drag on the plate diagram to select wells of interest.
2. Specify a Well Type category by selecting a Well Type icon above the diagram or by right-clicking after selecting the wells of interest. (See description below.)
3. Click **Save Experiment**.

Figure 3. Select the Well Type category.

> **NOTE**
>
> You may change the name of the plate at this point by right-clicking on the plate name you want to change, and selecting **Edit Plate Number**.

Well Types and Sample Categories

Immediately after clicking on a column, row, or a well-type icon, wells display the color corresponding to the selected sample category. The wells are tagged with the identifiers that can be used for further analysis in the Analysis tab.

Empty – The well will not be sampled.

Sample – A well is sampled and data are given the tag Sample.

Positive – A well is sampled and data are given the tag Positive.

Negative – A well is sampled and data are given the tag Negative.

Rinse – A well contains a rinse fluid, is sampled, but the well will not be identified for analysis.

Changing Default Colors in the Design Tab

The default colors for well assignments in the Design tab may be changed if desired for each individual experiment. Any changes made to the Well Type colors remain associated with the specific experiment only, and any new experiments will revert to the default colors.

1. In the Well Type menu bar, select **Customize**.
2. The Edit Annotation Layer window appears. Choose the well type to be changed and select **Edit**. Click on the colored circle.
3. The Select Color window appears. Left-click in the spectrum to select the desired color, or, change the color by adjusting the hue, saturation, brightness, and/or the red, green, and blue values below the spectrum.
4. Select **OK** to finish. It is now possible to choose another Well Type to change colors, following the previous three steps. The new category color now appears in the top menu.

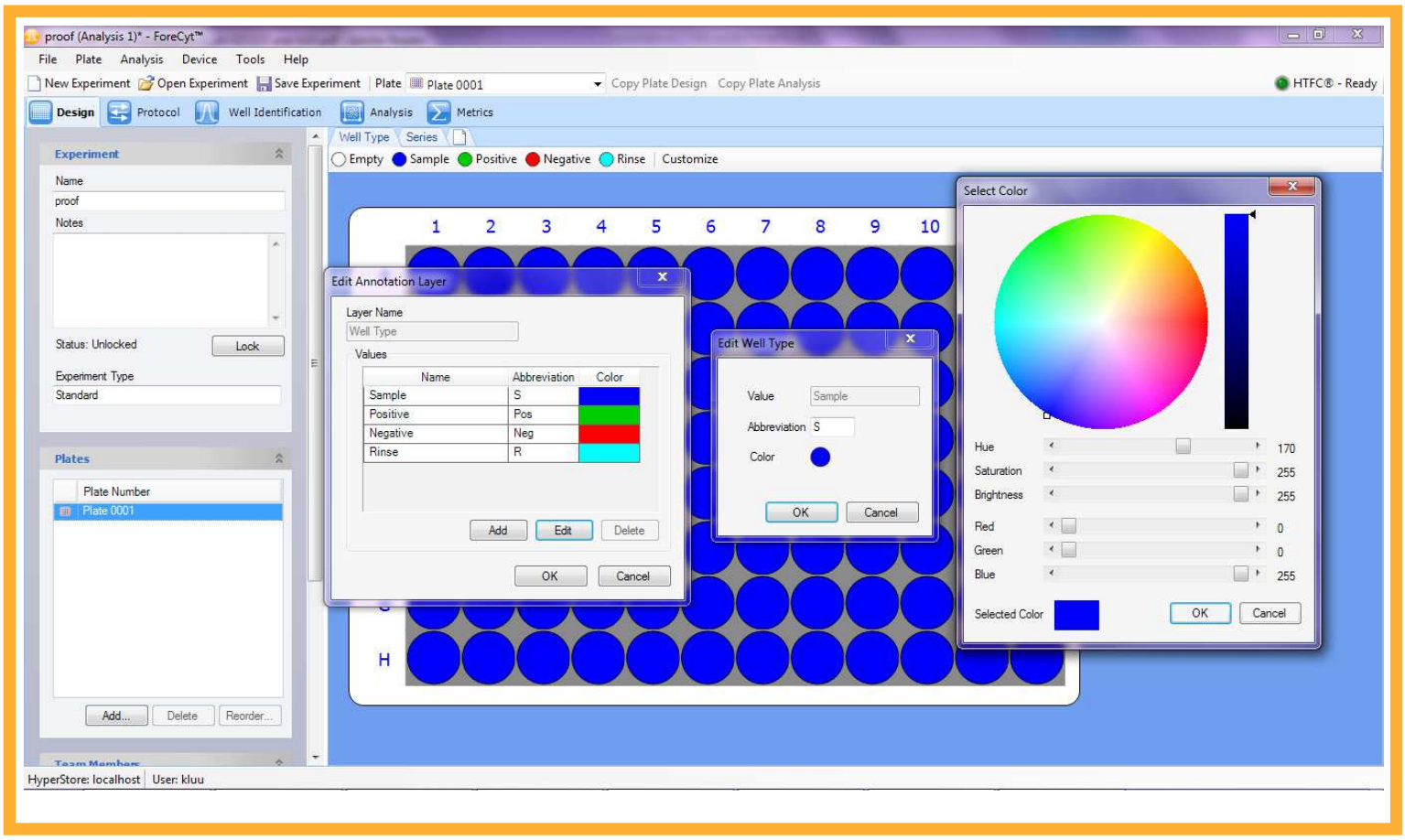

Figure 4. Picking a color from the spectrum.

Adding a Series

Visit the link below to watch a video tutorial on Adding a Series.

http://www.intellicyt.com/training/dose-response-curves

Add Well Notes

A user may add or annotate any well-specific information as needed. This is useful for flagging any additional information or experimental observations that might not have been captured as a formal annotation.

1. Drag and select the desired wells
2. Right-click, and choose **Add/Edit Well Notes**.
3. Enter the desired well note information in the Add/Edit Well Notes window and select **OK**. Each noted well will display a yellow circle in the upper left quadrant.
4. Hover the mouse over the well with contents to view notes.
5. If necessary, clear well notes by selecting wells with well notes, right-clicking, and selecting **Clear Well Notes**.

Plate Management

Plates within multi-plate experiments may be individually changed or have other plate settings copied to them. To copy design and acquisition settings from one plate to others in the experiment:

1. Choose the **Copy Plate Design** button in the toolbar. This function will bring up the Copy Plate Design window.
2. Using the checkboxes, choose which plates the settings will be copied to, and which specific features should be copied.

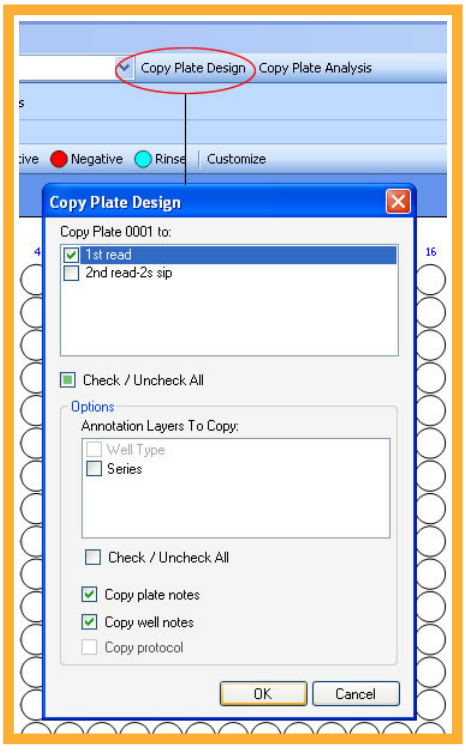

Figure 5. Choose Copy Plate Design.

Protocol

The Protocol tab creates the experimental settings that define how the sampler acquires test samples from the plate. The Protocol tab contains the following panes:

- **Prepare** controls how the sampler prepares the peristaltic tubing and sample plate before any test samples are acquired.
- **Sample** controls sampling order, duration, speed, and plate model.
- **Rinse** controls how often the probe is rinsed in the rinse solution.
- **Shake** controls the shaking of the plate to maintain the well contents in suspension.
- **Flush and Clean** controls cleaning of the probe at the end of the experiment.
- **IntelliCyt Cytometer** controls the acquisition threshold settings.
- **Worklist** displays a preview of the sequence of events the sampler will perform based on the protocol specified in the other panes.

CAUTION

Improper setting of these thresholds may result in loss of data. IntelliCyt highly recommends to leave these settings at their default values, or to use the assay templates provided for specific reagent kits. For additional guidance on assay setup and optimizing protocol settings, refer to Q-Guide: Assay Best Practices

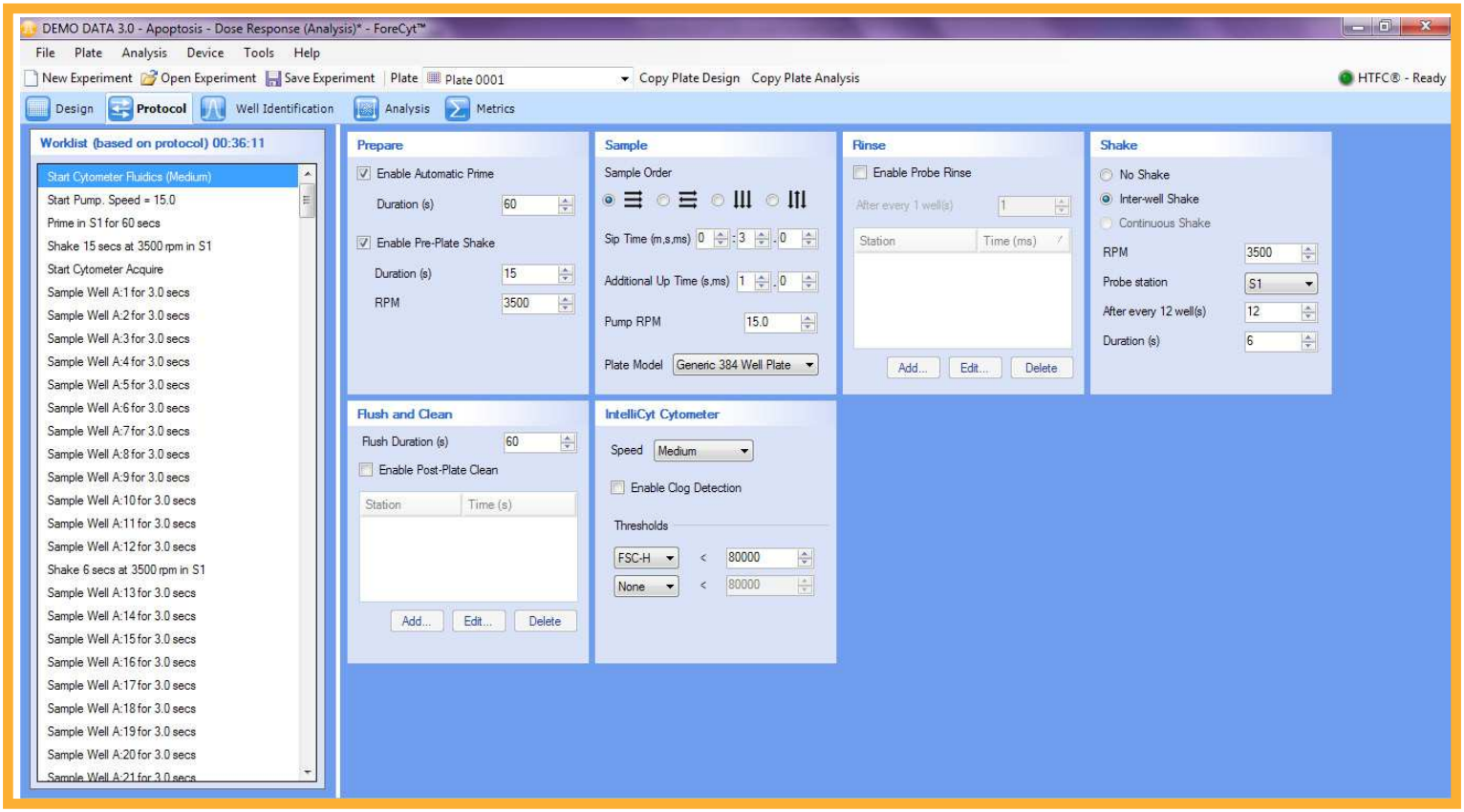

Figure 6. The Protocol Tab.

Prepare

In the Prepare pane, the user may select optional steps for the sampler to perform that may aid in sampling robustness.

Figure 7. Prepare Pane.

Check Enable Automatic Prime.
Define the duration in seconds:
The duration specifies the total time for the prime step. The prime will utilize the buffer rinse station S1, and the same sip time/ up time specified in the experiment protocol. For example: if the experiment is set for a 4 second sip time, the prime will likewise sample for 4 seconds in S1 buffer.

Check Enable Pre-Plate Shake to mix the plate contents prior to beginning acquisition.

After the Automatic Prime is complete, the orbital shaker will perform a plate shake with the specified duration and speed. During the shake the sample probe will continue to prime in the S1 Rinse Station.

Select the Duration:
Enter the amount of time in seconds to mix the plate/samples adequately. The optimal duration of the shake varies by plate model and sample type. This field defaults to 15 seconds.

Select the RPM of the orbital shaker:
This field defaults to 2400 RPM (3000 for 384-well plates), which is optimized for up to 50µL/well in a 96-well plate and 30µL/well in a 384-well plate. If the assay plate contains considerably more volume per well, the shake speed should be lowered accordingly to avoid sample loss.

Sample

This pane is where the settings for sampling the wells are specified. Type or toggle the arrows next to Sip Time, Additional Up Time, and Pump RPM to adjust the values for these parameters. Select a Plate Model from the list. If the desired plate model does not already exist, a new model can be defined under **Device>Manage Plate Models**.

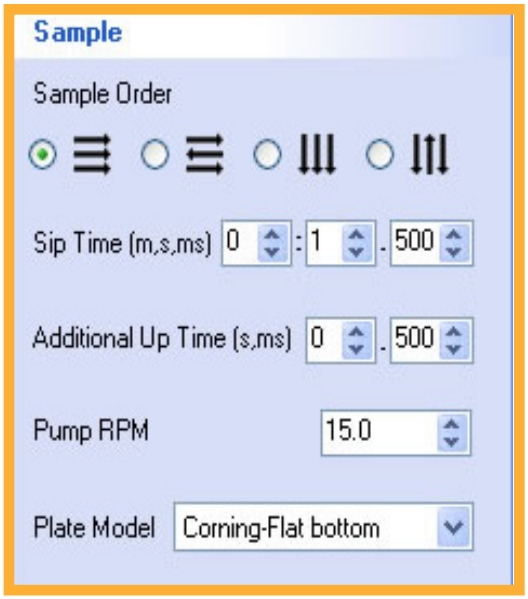

Figure 8. Sample Pane.

1. **Define Sample Order.**
   Click the radio button next to the diagram that indicates the order in which the sample probe samples the plate.
2. **Choose Sip Time and Additional Up Time.**
   The amount of time in each well, in conjunction with the pump speed, determines the volume of sample that is acquired. The minimum allowed Sip Time is 500 ms; the maximum is 60 minutes. Additional Up Time is the time the sample probe pauses above the well and affects the size of the air gap between samples.
3. **Set the Pump RPM.**
   This speed controls the rate that the peristaltic pump moves fluid through the sample line. The default value is 15 RPM, which will draw approximately 1-2µL/second from the sample wells. Lower pump speeds can be used to provide higher resolution data. Higher pump speeds deliver higher volumes, but decrease the data resolution. Allowable pump speeds are between 0.1 and 48 RPM.
4. **Select the Plate Model.**
   Select the desired plate model. If it is not on the list, define a new one using Manage Plate Models in the Device menu.

NOTE

Setting of additional up time is optional. The additional time has the effect of enlarging the air gap, as even with the minimum allowed value for additional up time set at 0 ms, a significant air gap will be formed.

Rinse

Defines the option to rinse the probe periodically at designated rinse station(s) during sampling.

- **Enable Probe Rinse** – Select the box to activate a rinse.
- **After every __well(s)** – Type or use the up and down arrows to increase or decrease the number of wells sampled between probe rinses.
- **Station** – After the selected number of wells, the probe cycles through each rinse station from the list, in the order they are listed. To insert a new rinse station, select **Add**. An **Add Rinse** window appears with station placement options. Alternatively, select a station from the rinse pane and **Edit** the rinse time.

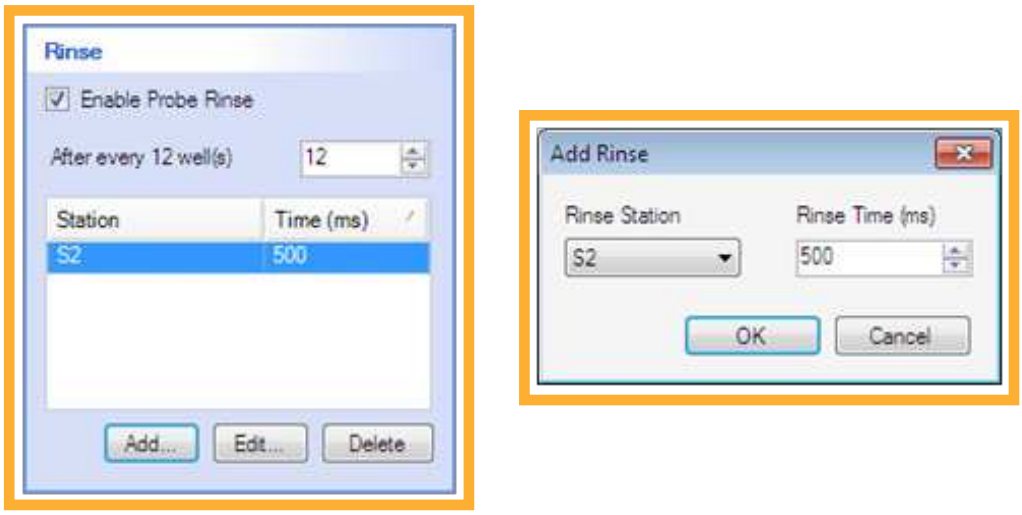

Figure 9. Adding rinses to be performed during a sample run.

A suggested list of rinse solutions for each station is as follows:

S1: Sample buffer (IntelliCyt strongly recommends the same buffer as used to prepare test samples)

S2: Decontamination Solution (IntelliCyt Cat # 90077)

S3: Cleaning Solution (IntelliCyt Cat # 90079)

S4: Filtered, deionized water

Fill each of these station containers to the very top with the appropriate fluid.

Shake

This controls the orbital shaker during the sampling run. During the period of time that the shaker is activated, the sample probe travels to a designated Probe rinse station and will continue to prime for the duration of the shake.

Figure 10. Shake Pane.

1. **Select a shaking mode:**
   No shake indicates that no mixing steps should occur during the plate acquisition.
   Inter-well Shake allows the plate to be shaken intermittently after a given number of wells.
   Continuous Shake enables shaking from the beginning to the end of the sampling run.
2. **Enter RPM for the Orbital shaker:**
   Set the RPM of the orbital shaker by using the up and down arrows or by typing the desired number in the box. The recommended RPM will vary based on plate type, well shape, and well volume, however, the default for 96-well plates is 2400 RPM and for 384-well plates is 3000 RPM.
3. **Select Probe station:**
   This defines which rinse station location to utilize during an inter-well shake. Select rinse stations S1 – S4 from the drop-down menu.
4. **Select frequency to rinse – After every __ wells:**
   Type or use the up and down arrows to select the number of wells acquired between shakes.
5. **Select Duration (of the shaking) in seconds:**
   Enter a number or use the up and down arrows to choose how long to shake.

NOTE

As the probe undergoes a rinse and prime during the shake, it is often unnecessary to define a separate rinse step.

Flush and Clean

After the samples have been acquired from the assay plate, they travel within the sample tubing to the detector. Based on the tubing length and pump speed, this process can take from ~30 seconds (at 15 RPM) to upwards of several minutes at lower RPMs. The flush is designed to ensure that all samples are delivered to the cytometer for analysis before the pump turns off.

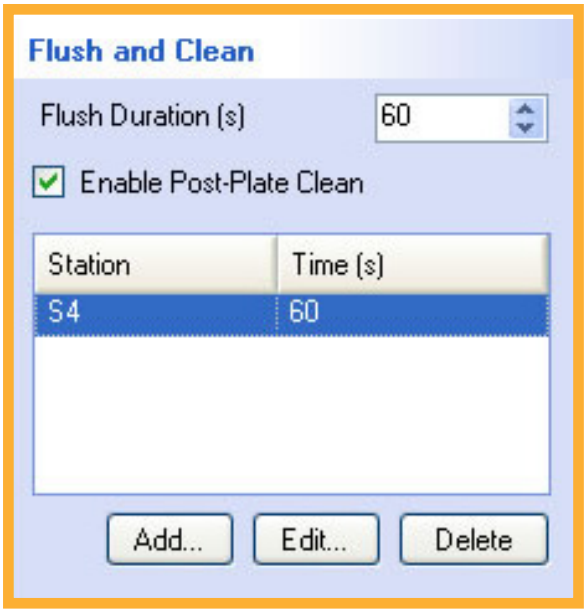

Figure 11. Flush and Clean Pane.

1. **Select the Flush Duration**
   In order to ensure that all the sample slugs have passed through the peristaltic tubing and into the cytometer, a flush at the end of sampling is mandatory. The default flush is 60 seconds, with a minimum flush duration of 30 seconds. IntelliCyt strongly recommends a flush of 60 seconds unless a Post-Plate Clean is selected (see below, step 2).

   During the flush, the sampler probe will cycle in the S1 Rinse Station according to the sampling protocol.

NOTE

If the pump RPMs are set for lower than 15 RPM, the flush duration will need to be increased to ensure complete data acquisition. For lower RPMs, the flush time can increase to as much as 180 seconds. Please ensure that proper values are set as to prevent data loss.

2. **To Enable Post-Plate Clean**
   Post-Plate Clean is optional and the decision to use it depends on sample type, or duration of sampling the plate. To perform this function, use the **Add** button to configure which rinse station(s) the probe will draw from at the end of the plate.

   The Add button allows the user to designate a rinse station(s) from the list of S1-S4, and enter a period of time in seconds.

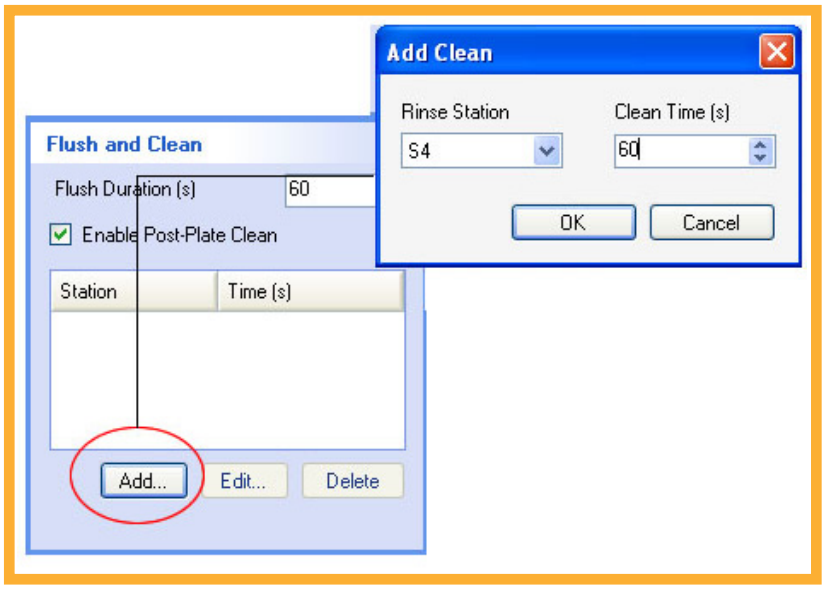

Figure 12. Customize Post-Plate Clean.

NOTE

All IntelliCyt Screening Systems, including the iQue Screener and HTFC, should be fully cleaned at least daily.

IntelliCyt Cytometer

The IntelliCyt Cytometer pane offers several options for sample acquisition. The speed of the cytometer sheath fluid affects the size of the sample core and potentially data resolution. The threshold settings establish cut-off values below which data are not acquired, and can be set on either size (FSC) or any of the fluorescence channels. It is important to note that changing these values will determine what data are acquired, and improper adjustment of these values could result in data loss. IntelliCyt highly recommends the use of default threshold settings for all general experiments.

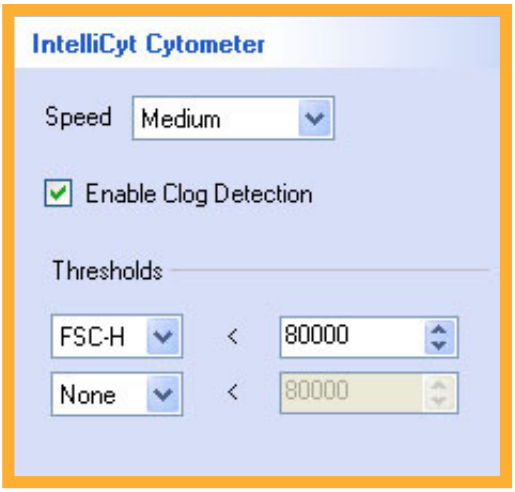

Figure 13. IntelliCyt Cytometer Pane.

Save Experiment

After completing the experiment design, select the **Save Experiment** icon or choose **File** in the menu bar and then select **Save Experiment** or **Save Experiment As** from the drop-down menu. If an experiment has unsaved changes, an asterisk (*) will appear next to the name of the experiment in the header bar of the window.

Save Experiment As

A new experiment may also be created by opening a previously-run experiment and choosing **Save Experiment As.** **Save Experiment As** creates a duplicate of the original experiment, complete with settings and analysis features.

Open Experiment

Use the **Open Experiment** button to browse for previously-created experiments. Select **File>Recent Experiments** to see a list of recently used experiments.

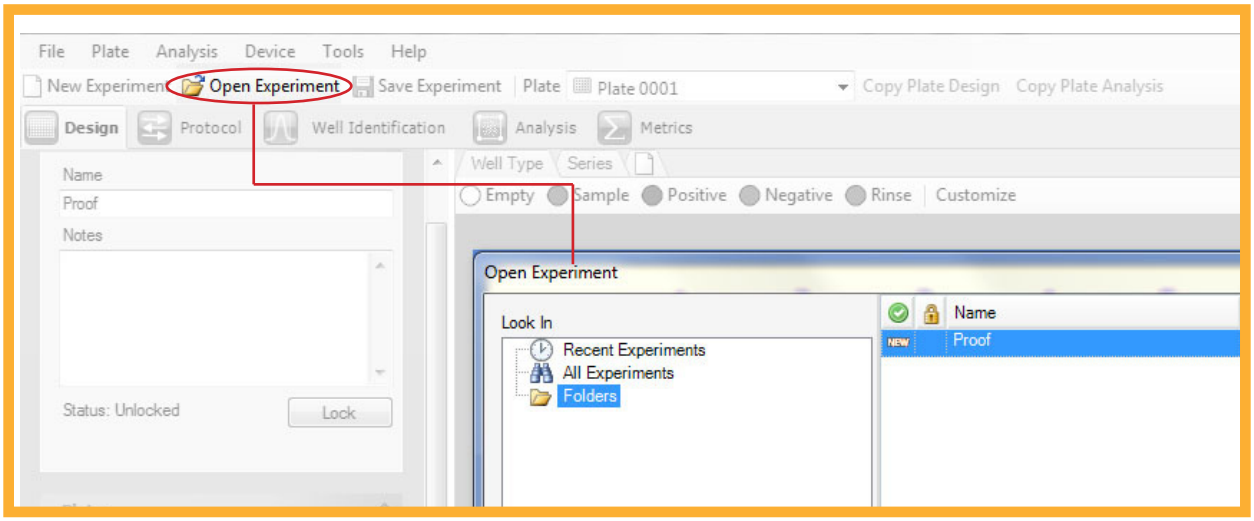

Figure 14. Opening a recent experiment design.

Acquiring Data Samples: Controller

The Controller manages acquisition of sample plates, and the status of all devices. It is located in the upper right-hand corner of the grey title bar. The Controller consists of the following panes:

- **Controller Buttons (Prime/Run/Clean/Stop):** these buttons designate the mode in which the system is operating.
- **Device Details Tab:** lists the status of each device, including the components of the sampler, as well as the cytometer.
- **Run Progress Tab:** contains the step-by-step protocol that will be followed as the plate is sampled. The wells will highlight as they are acquired by the sampler.

Controller Buttons

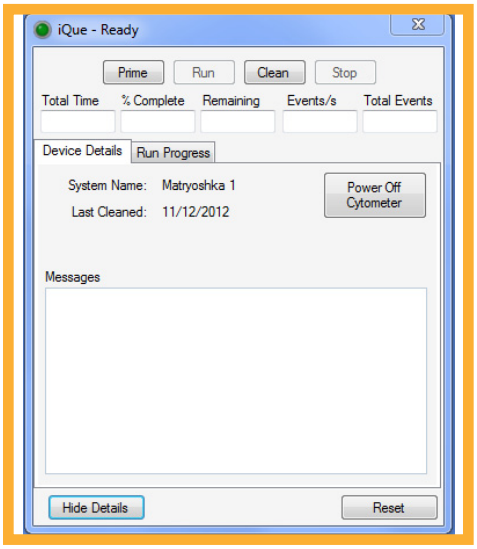

Figure 15. Controller

- **Prime:** allows the sampler to alternately sip buffer from the S1 Rinse Station and air, without recording data. Prime mode will mimic the sampling settings defined in the Protocol tab if an experiment is active. If no experiment is open, the Prime mode will default to a 1-second sip, 1-second up method. In Prime mode, the cytometer fluidics will also switch on. Ensure that the tubing has been attached to the sample inlet. During an acquisition run, this button is disabled.
- **Run:** initiates the run as specified by the experiment protocol settings. If no experiment is loaded, this button is disabled.
- **Clean:** allows the user to initiate the sampler cleaning protocol. During an acquisition run, this button is disabled.
- **Stop:** during a run, this button allows the user to stop the run before the Worklist has completed. The user will be prompted to attach any already-collected data. This button is only enabled during an acquisition run.

Device Details

A green light in the title bar, and the message “Ready,” indicates that all components of the system are connected and working appropriately.

A yellow light in the title bar, and the message “Warning,” indicates that there may be a problem with one of the components. For details and instructions, select the **Show Details** button. Once the problem is identified and fixed, select the **Reset** button to reset the system.

A red light in the title bar, and the message “Not Ready,” indicates there is a major problem with one of the components of the system. This message is typically seen when one of the components is not powered. Select the **Show Details** button to see which component is causing the error.

Run Progress

The Run Progress pane displays the step-by-step protocol of how the sampler will behave during data acquisition. As a step is completed, it will be checked off in the list. The current step will highlight as it is being performed.

The Run Progress pane is a duplicate of what was chosen in the Design tab. It displays the location of the Sample, Positive, Negative, and Rinse wells. When an acquisition run is initiated, all wells will dim. As each well is sampled, the well will highlight. Once the well acquisition is completed, the vibrant well color returns to indicate the completed acquisition of the well.

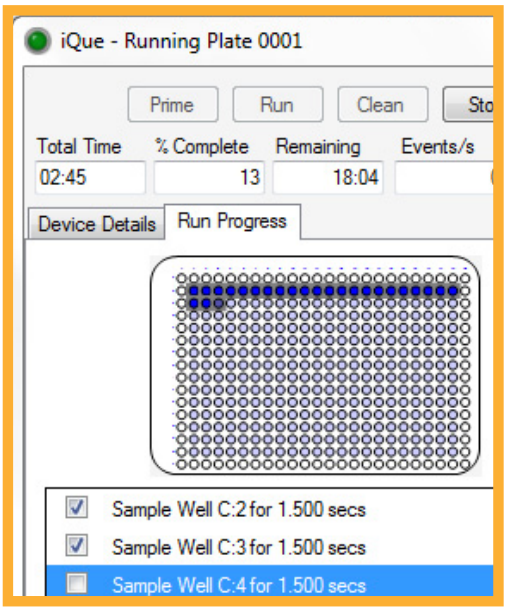

Figure 16. Run Progress Pane

Sampling a Microplate Using Controller

NOTE

Before running an experiment, calibrate the x,y,z positions for the sample probe. Recalibrate after changing the sample probe or if using a different plate model or different computer.

Select Plate and Start Controller

1. Load your plate onto the shaker platform, taking care to ensure the plate is in the secure position. Make sure sample well A1 is in the upper left corner.

CAUTION	Verify that the plate model selected in the experiment protocol corresponds to the model being used, and that the plate model is correctly calibrated for the desired sampling depth.

2. Choose the desired plate for acquisition. This can be achieved using the drop-down menu from the taskbar, or by selecting the plate within the Protocol tab.
3. Select the device name in the upper right corner to bring up the Controller.
4. If the Controller signals Warning (as indicated by a yellow light) or Not Ready (as indicated by a red light), select the **Show Details** button to view the status of each of the devices.
5. If any device fails to display green, check the connections and press **Reset**.
6. When all devices are ready, prime the sampler if desired, then select **Run** to begin the acquisition.

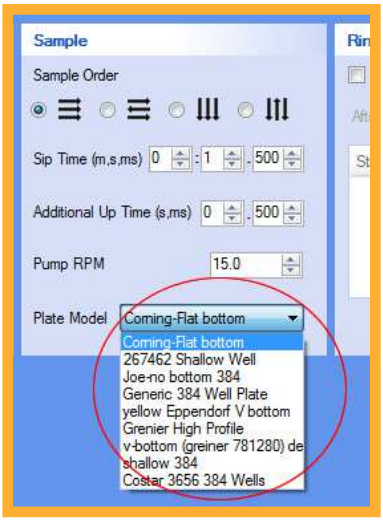

Figure 17. Select Plate to Run.

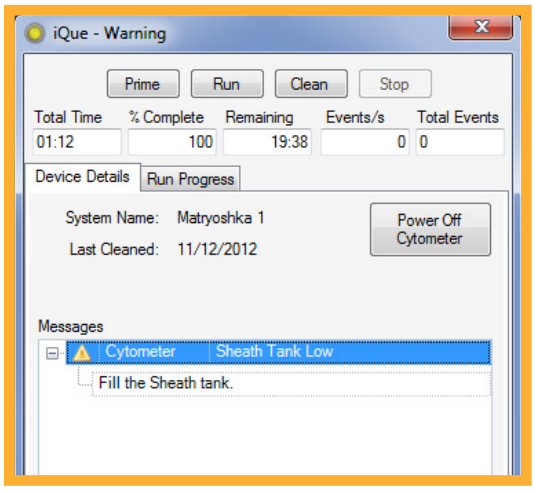

Figure 18. Controller

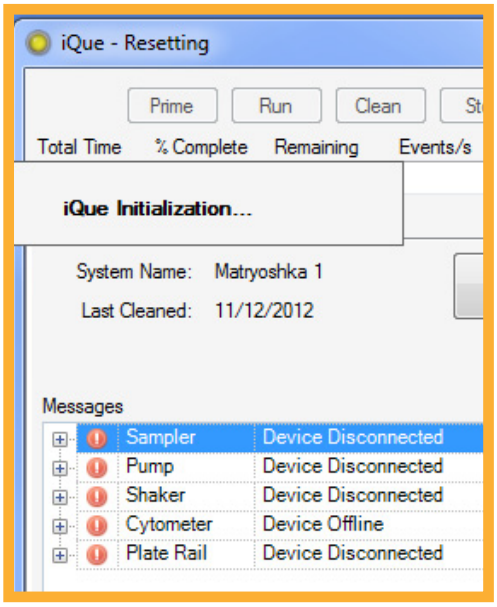

Figure 19. iQue Initialization

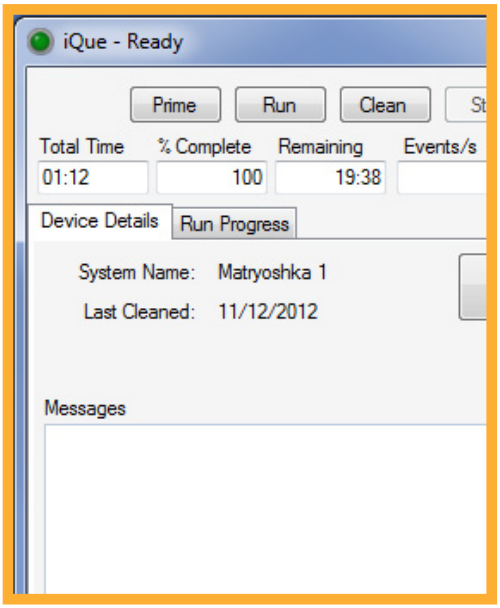

Figure 20. Run Experiment.

Running a Multiplate Experiment

If the experiment contains more than one plate, the user must select the next plate for acquisition.

1. Select the next plate in the menu bar. Additionally, the next plate may be selected in the list in the **Design** tab. Plates that have been acquired, and therefore have FCS data attached, have a green check mark icon. Plates that have not been run yet have a plate model icon.

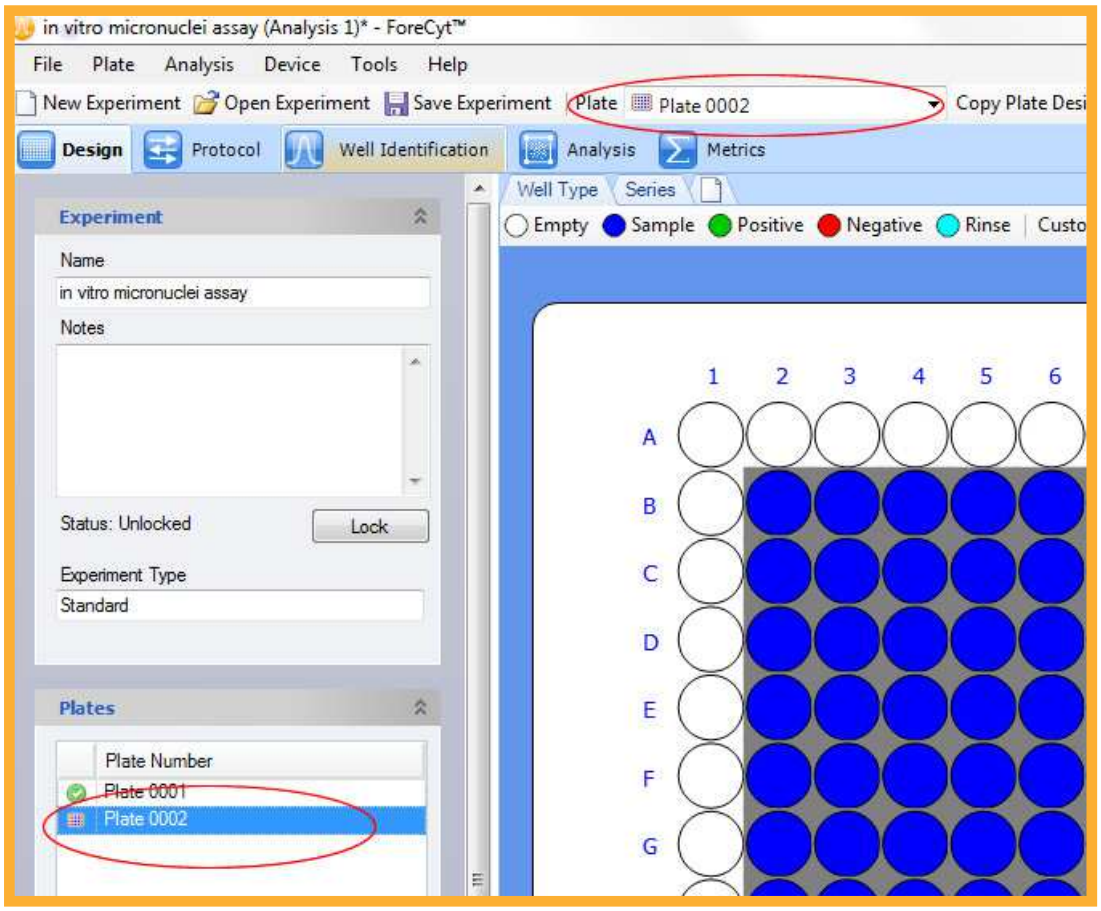

Figure 21. Switching Plates Within an Experiment.

2. Select the device name in the upper right corner to bring up the **Controller**.
3. Select **Run** to sample the plate.
4. Repeat for all remaining plates in the experiment.

NOTE

In a multiplate experiment, the plates may be acquired in any desired order.

Completion of Run

Once a plate has been sampled, the resulting FCS data will automatically attach to the experiment. Now the user may continue on to analyze the data for the plates. Refer to appropriate videos and Q-Guides for additional information on identifying wells and data analysis.

Clearing FCS Data

On occasion, it may be necessary to clear FCS data from a plate:

1. In the **Design** tab, right-click on the name of the plate from which data will be cleared, and choose **Clear FCS Data**. This may also be achieved by selecting **Plate** in the menu bar and choosing **Clear FCS Data**.
2. Verify that this is the correct choice, as the software will permanently delete the data from the plate.
3. The plate is now cleared of FCS data and may be re-acquired.

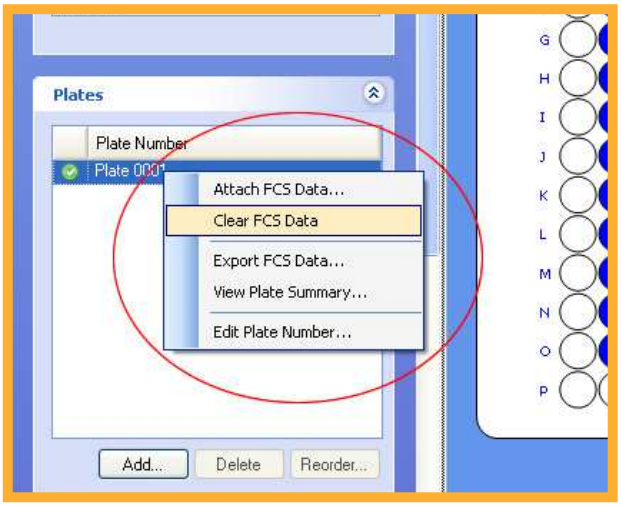

Figure 22. Clear FCS Data.

CAUTION

Data deletion is permanent.