Luminex®

Software Quick Guide

xPONENT® for FLEXMAP 3D® Version 4.2
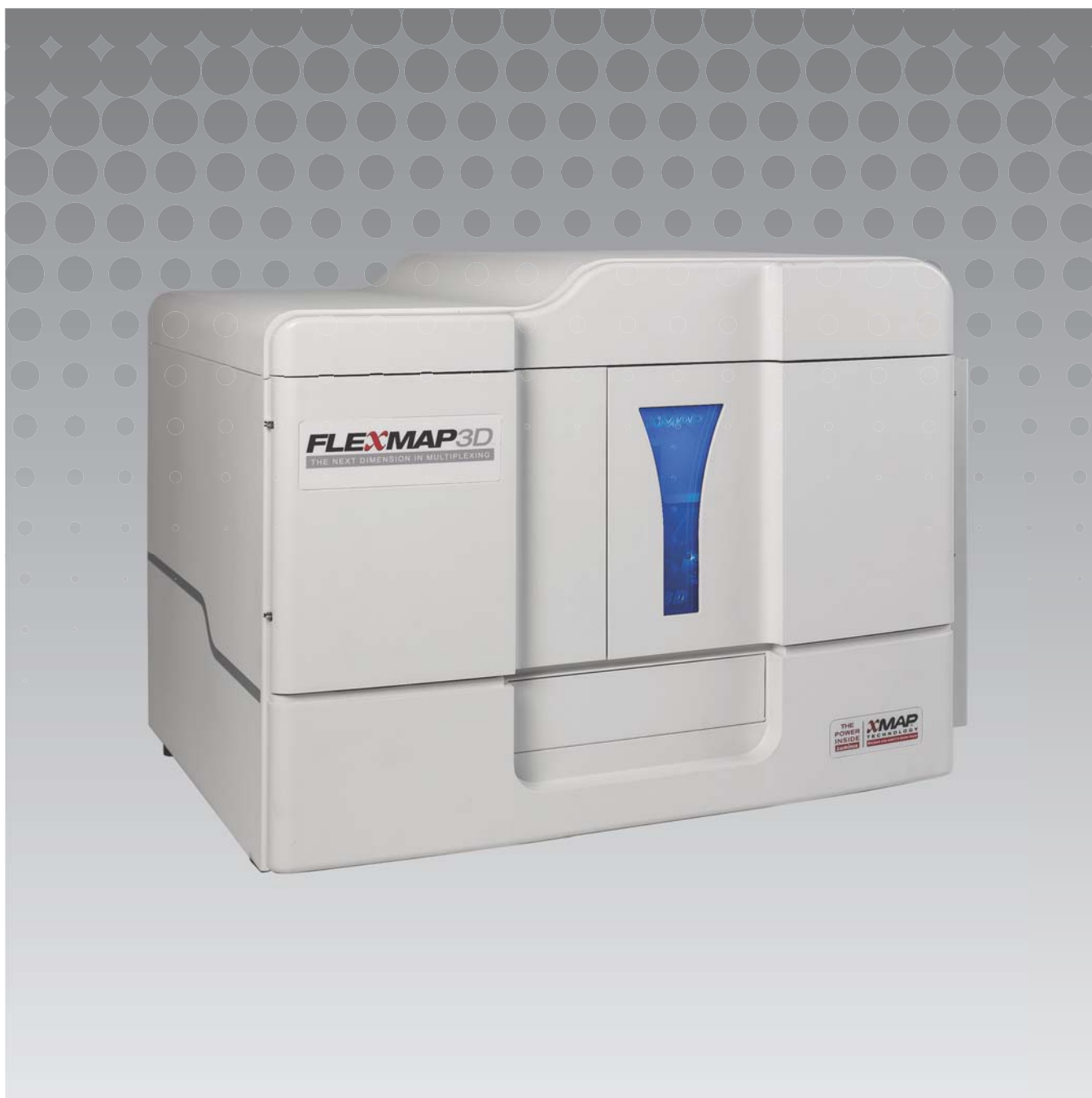
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xPONENT® for FLEXMAP 3D® Software Version 4.2 Quick Guide

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Table of Contents

Starting xPONENT®

To start xPONENT®:

- On the PC desktop, click the Luminex® xPONENT icon, or click **Start > All Programs > Luminex > xPONENT > Luminex xPONENT**.
- If you have a trial license, contact Luminex "*Technical Support"* to obtain a full license, or click **OK** in the dialog box to continue.
- If this is the first time you have started the software, the **End User License Agreement** may display. Read the license agreement. Select **I accept the terms of this license agreement**, then click **OK**.

  NOTE: For safety and legal information, refer to the *xPONENT® for FLEXMAP 3D® Hardware Version User Manual* that you received with your instrument.

Start-up Indicators

When you turn on the Luminex® FLEXMAP 3D® instrument, the following start-up indicators occur:

- The blue light and power supply fan turn on.
- The compressor and MAC valve cycle on (MAC valve makes a distinct sound).
- Immediately, the sample valve cycles (distinct sound).
- Immediately, the probe actuator cycles.
- Immediately, the right syringe pump takes a stroke.
- Immediately, the exhaust fans turn on.
- The left syringe pump takes a stroke.
- Drip to waste occurs.
- The sample valve cycles again approximately 15 seconds to 30 seconds after the left syringe pump completes stroke.

Common Daily Activities

This flowchart illustrates the flow of common daily tasks. For more information about any task, see the *Luminex® FLEXMAP 3D® Installation and Hardware User Manual* or the *xPONENT® for FLEXMAP 3D® Software User Manual*.

FIGURE 1. **Common Daily Activities Flowchart**

```
                    ┌─────────────────────┐
                    │   Start Software     │
                    └─────────────────────┘
                              │
                              ▼
                    ┌─────────────────────┐
                    │  Adjust Probe Height │
                    └─────────────────────┘
                              │
                              ▼
        ┌─────┐        ◇ Are you using new ◇        ┌─────┐
        │ Yes │◄───────◇      kits?        ◇───────►│ No  │
        └─────┘        ◇                   ◇        └─────┘
           │                                           │
           ▼                                           │
  ┌──────────────────┐     ┌──────────────────┐        │
  │ Import Kit       │────►│ Select Correct   │◄───────┘
  │ Information      │     │ Kit Lot          │
  └──────────────────┘     │ Information      │
                           └──────────────────┘
                                    │
                                    ▼
        ┌─────┐        ◇ Does the instrument ◇       ┌─────┐
        │ Yes │◄───────◇   need to be         ◇─────►│ No  │
        └─────┘        ◇   calibrated?        ◇      └─────┘
           │                                           │
           ▼                                           ▼
  ┌──────────────────┐                        ┌──────────────────────┐
  │ Run System       │                        │ Run "Performance      │
  │ Initialization   │                        │ Verification" from the│
  └──────────────────┘                        │ Auto Maintenance page │
           │                                   └──────────────────────┘
           │              ┌──────────────────┐         │
           └─────────────►│ Collect Data:    │◄────────┘
                          │ Create Protocol, │
                          │ Lots and Kits,   │
                          │ Batches          │
                          └──────────────────┘
                                    │
                                    ▼
                          ┌──────────────────┐
                          │  Analyze Data    │
                          └──────────────────┘
                                    │
                                    ▼
                          ┌──────────────────┐
                          │ View and Print   │
                          │ Reports          │
                          └──────────────────┘
                                    │
                                    ▼
                          ┌──────────────────┐
                          │ Daily Shutdown   │
                          └──────────────────┘
```

Software Overview

xPONENT® software is organized into a series of pages. As you click each page, applicable tabs open on the left-hand side of the window.

xPONENT opens to the **Home** page. You can access most tasks from the **Home** page.

xPONENT includes the following pages:

- **Home** - Access most commonly used features
- **Samples** - Import or enter sample data (optional)
- **Batches** - Set up batches
- **Results** - Analyze previously acquired batches; view current and saved reports
- **Protocols** - Displays protocols, Stds & Ctrls, and Lot information
- **Maintenance** - Calibrate, verify, and maintain the application
- **Admin** - Perform administrative tasks
- **Log Off** - Log out of software
- **Help** - Access help file

FIGURE 2. **xPONENT® Home Page**
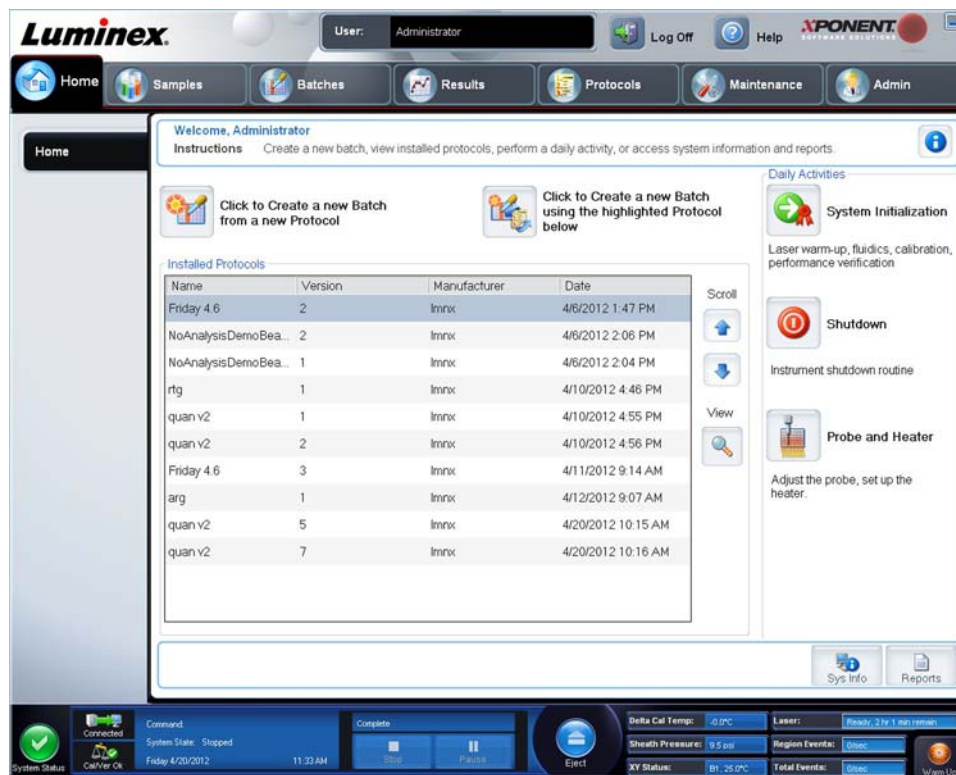
System Monitor

The **System Monitor** is displayed at the bottom of all xPONENT® windows. It displays the physical state of the Luminex® system. Values are reported directly from the Luminex system.

FIGURE 3. **xPONENT® System Monitor**
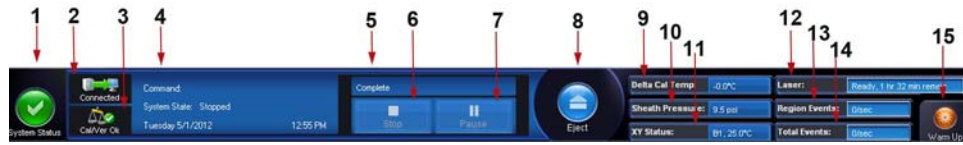
1.	System Status button	6.	Stop button	11.	XY Status
2.	Connection Status	7.	Pause button	12.	Laser Status
3.	Check Cal/Ver Ok	8.	Eject button	13.	Region Event Status
4.	Command Display	9.	Delta Cal Temp Status	14.	Total Events Status
5.	Progress Bar	10.	Sheath Pressure	15.	Warm Up button

System Status Button - This button has two functions: When clicked, it opens the system log. It also displays the current status of the system. If there are no warnings or errors, the **System Status** button is green with a check mark. If there is a warning, out of calibration condition, or other important user notification, the button is yellow with an exclamation point.

Connection Display - Displays the connection status.

Cal/Ver Ok - This will be lit up if the calibrations and verifications are connected. When you click the **Cal/Ver** button, it takes you to **Maintenance > Auto Maint**.

Command Display - Displays the following:

- The command currently running
- The system state (i.e., running, idle, etc.)
- Date and time

Progress - Displays a bar graph showing the progress of the current command or routine; if the command or routine is finished, it displays a full progress bar and the command status as **Complete**.

Pause - Pauses the system after the current command completes. **Pause** does not stop the system in the middle of running a command. You cannot run another command while the system is paused. Pause the system before stopping it so that it will finish the current command, store the pending batch and then resume exactly where it left off.

Stop - Stops the system, regardless of command status. Use this only if it does not matter whether the data from the current well is lost.

Eject - Ejects the plate. Once the plate is ejected, the **Eject** button changes to **Retract**. **Retract** retracts the plate, and the **Retract** button changes back to **Eject**.

Delta Cal Temp - Displays the difference in temperature in degrees Celsius between the current reading and the reading when the system was calibrated. If the temperature is out of tolerance, this shows a high or low arrow. When clicked, it opens the **Auto Maint** tab.

Sheath Pressure - Displays the sheath pressure in psi. A high or low arrow is displayed if the pressure is trending up or down versus the calibration pressure it turns yellow. When clicked, it opens the **System Info** tab.

XY Status - Displays the current location of the command and the temperature of the plate heating block in degrees Celsius. When clicked, it opens the **Probe & Heater** tab.

Laser Status - Displays the laser status, including the time remaining until you must warm up the laser again. The **Laser status** box is blue. The button turns yellow when the lasers are turned off and about 10 minutes before they turn off. Clicking the **Warm Up** button restarts the active clock for the laser.

Region Events Status - Displays the number of bead events detected per second that are classified in a region.

Total Events Status - Displays the number of total events detected per second.

Warm Up Button - Starts or schedules a warm up.

Initial Startup

When you turn on the system for the first time, perform the following procedures:

1. "*Adjusting the Sample Probe Height*"
2. "*Initializing the System*"

Adjusting the Sample Probe Height

Adjust the sample probe height to ensure that the probe drops far enough into the well to acquire sample.

NOTE: Ensure that there is no liquid in the wells or reservoirs before adjusting the sample probe height.

NOTE: When you adjust and save the probe height settings for all three areas under a plate name, all areas retain the adjustment.

> **WARNING:** Correct sample probe height is critical to successful sample acquisition and calibration. Problems with the sample probe height can lead to fluid leaks and inhibit sample acquisition.

1. On the **Home** page, click **Probe and Heater** under **Daily Activities**. The **Probe & Heater** tab opens.
2. Select a plate in the **Plate Type** list.
   - **96-well and 384-well Hard Bottom Plates** - No disk is required. Ensure that the well location is selected on the plate image. Use well **D6** for the 96-well plate and well **H12** for the 384-well plate (a green pin marks the location). To change the well location, click on the desired well in the plate image.
   - **96-well, 384-well Filter, or Mylar Bottom Plates** - Use the Probe Height Adjustment Tool (not supplied).
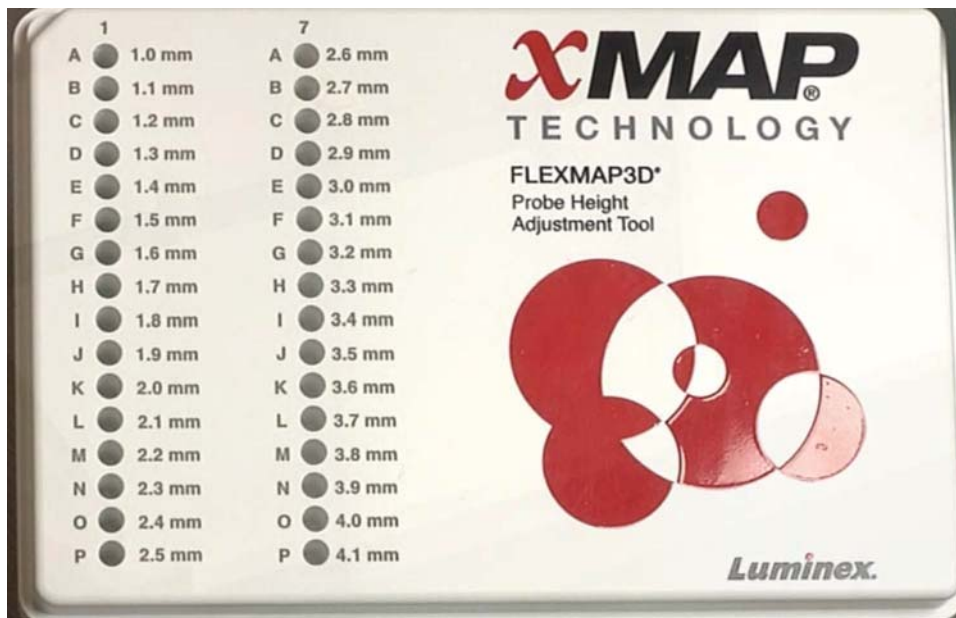
> **NOTE:** You can order the Probe Height Adjustment Tool (P/N CN-0298-01) at *http://www.luminexcorp.com*.

3. Verify that the plate is not warped. Warped plates can lead to incorrect probe height adjustment.
4. Click **Eject** to eject the plate carrier.

> **NOTE:** If using a strip-well plate, ensure that a strip is present in the selected well location, prior to calibration.

5. Place the plate on the plate holder with **A1** in the top left corner. Place the reagent block on the plate carrier. Place a well-strip (provided with the Calibration and the Performance Verification kits) in column **S1** of the block.

> **NOTE:** Make sure there is no liquid in the plate or the off-plate reagent block.

6. Click **Retract** to retract the plate carrier.

7. Type a name for the plate in the **Plate Name** box, or choose a saved plate from the **Plate Name** drop-down list.

> **NOTE:** If a saved plate is selected the results of the new calibration will override the previous calibration.

8. In the **Plate** section, click **D6** for the 96-well plate. Click **H12** for the 384-well plate.

9. In the **Reservoir** section, click **RB1**.

10. In the **Strip-Wells** section, click **SD1**.

11. Click **Auto Adjust Height**. The probe automatically adjusts itself and saves it to the plate you selected.

> **NOTE:** The probe height is automatically set to 0.49 mm. The probe automatically adjusts this distance from the bottom of the plate or calibration disks.

> **NOTE:** When you adjust and save the probe height settings for all three under a plate name, all areas retain the adjustment.

WARNING: Correct sample probe height is critical to successful sample acquisition and calibration. Problems with the sample probe can lead to fluid leaks and inhibit sample acquisition.

CAUTION: Ensure that the probe height is set correctly before calibrating the system.

Revive After Storage Routine

NOTE: The **Revive After Storage** routine is necessary when the system has been idle for more than a week.

After you have adjusted the sample probe height, run the **Revive After Storage (Luminex)** routine.

1. Open the **Maintenance** page, then the **Cmds & Routines** tab.
2. Choose **Revive After Storage (Luminex)** from the **Routine Name** drop-down list. The **Revive After Storage** routine performs the following commands:
   - **Warmup**
   - **Backflush (x2)**
   - **Drain RA2 (x3)**
   - **Alcohol Flush RB1 (x2)**
   - **Backflush**
   - **Wash RA1 (x3)**
3. Add 70% isopropanol or 70% ethanol to reservoir **RB1** on the off-plate reagent block as indicated on the **Cmds & Routines** tab. Add DI Water to reservoir **RA1**.

   NOTE: The drain reservoir (**RA2**) should be empty.

4. Click **Run**.

After the **Revive After Storage** routine is complete, run the **System Initialization** routine.

Initializing the System

xPONENT® enables you to customize system initialization using one of these three options:

- Laser warm-up, fluidics prep, calibration, performance verification
- Laser warm-up, fluidics prep, performance verification
- Warm-up fluidics prep

   NOTE: Verify the system daily and calibrate weekly to check system integrity and to ensure that calibration is still valid.

To run the system initialization routine:

1. On the **Home** page, click **System Initialization**.
2. Choose the performance verification kit from the **Performance Verification Kit** list.
3. Choose the calibration kit from the **Calibration Kit** list.
4. Vortex the xMAP® calibrator, verification, and fluidics containers for 30 seconds to ensure homogeneity. Do not dilute xMAP calibrator, verification, or fluidics agents.
5. Click **Eject** on the status bar.
6. Place the off-plate reagent block on the plate holder. Load a strip well into the block.
7. Load wells with at least 5 drops of the Calibration, Verification, and Fluidics beads, as indicated on the plate image and in the kit insert instructions.

   NOTE: To ensure you get the necessary bead count, invert the calibrator and verifier vials perpendicular to the plate as you add drops to the wells. This ensures the maximum fluid drop size is dispensed into the wells.

8. Click **Retract**.

9.   Click **Run**.
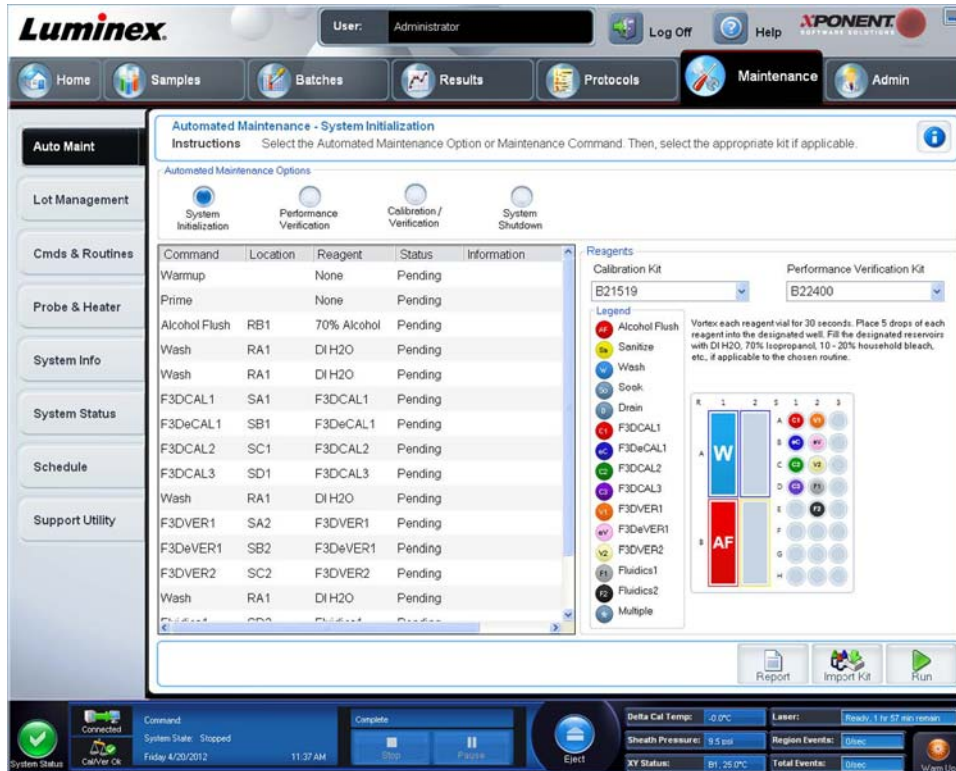
Running, Saving, and Printing Reports

xPONENT® can provide information in six different report types:

- Batch
- Protocol
- Calibration and Verification (Often used when troubleshooting CAL/VER failures)
- Performance Verification
- System Log
- Advanced (User)

xPONENT can also format your batch or multibatch results in a variety of export formats.

To run, print, and/or save a report:

1.   Open the **Results** page.
2.   Click the **Reports** tab.
3.   In the **Report** drop-down list, choose the report that you want to view.
4.   In the **Type** drop-down list, choose the type of report you want to view.

     NOTE: Select dates if applicable.

5.   Select the item for which you want to generate the report. If you are creating a **Batch Report**, select the analytes to include in the report.
6.   Click **Generate**.

7.	Click **Print** to print the report, or **Save** to save the report as a PDF file.

Creating a Protocol

You can create protocols with four types of analysis:

Quantitative - Analyzes unknown data generated with a standard curve.

Qualitative - Analyzes unknown data generated with cutoff ranges based on one standard.

Allele Call - Analyzes data generated as either heterozygotes or homozygotes, based on specific cutoff ratios.

None - Used when analysis of the data will be carried out in another program. (xPONENT® reports are unavailable for data acquired with a protocol of type "none".)

You can create each quantitative, qualitative, and allele call protocols using one product composed of a definable number of standards and controls.

There are three steps to creating a protocol. All three are accessible from the **Protocols** page, **Protocols** tab. The three steps are:

- Define settings
- Select the analysis type
- Define the plate layout

To create a protocol:

1.	Open the **Protocols** page.
2.	Click **Create New Protocol**. The **Settings** tab opens.
3.	Type the protocol Name, Version, and Manufacturer, and add an optional description in the empty field.
4.	Type the following information in the **Acquisition Settings** panel:
	- **Volume (10 µL to 200 µL)**
	- **XY Heater (35°C to 60°C)**
	- **Timeout (1 second to 250 seconds)**

- **Bead Type**
- **Plate Name**
- **DD Gating**
- **Reporter Gain**

5.   Choose the analysis type (**Qualitative**, **Quantitative**, **Allele Call**, or **None**), and enter the number of standards and controls.

6.   Select **Fit of all Standards** or **Mean of Replicates**.

7.   Select **Analyze results while acquiring samples** if you want to view real-time analysis.

8.   Click **Next** to open the **Select Analytes** tab. Select analytes by clicking the bead number. The table allows you to name the analyte and to select the analysis type, the units to be measured, and the count.
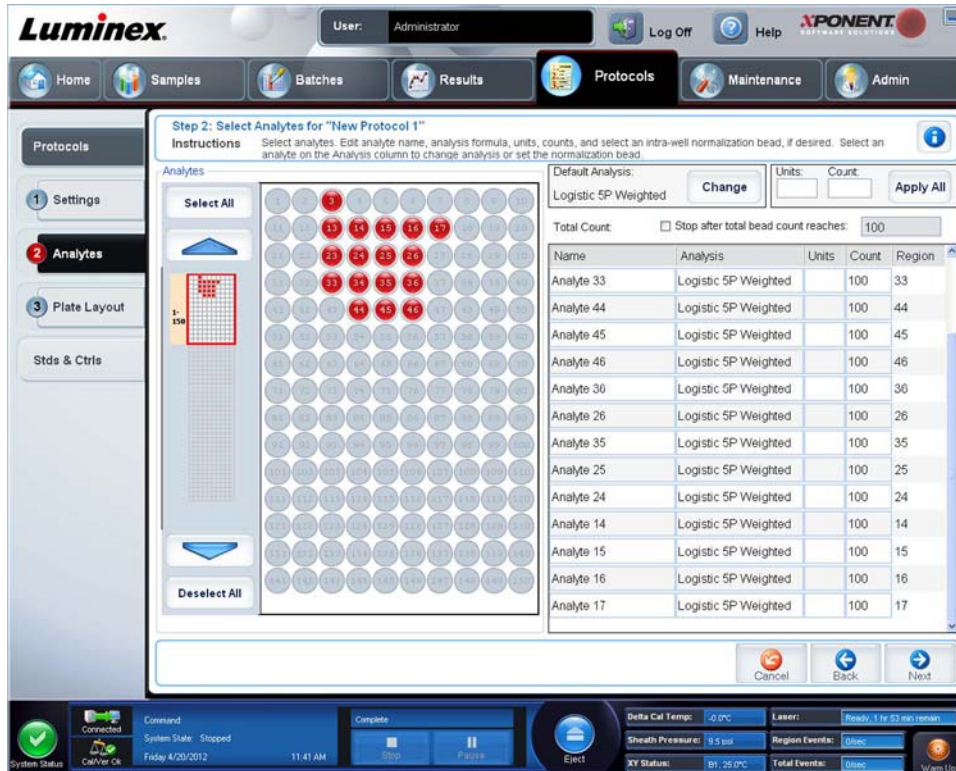
9.   Click **Change** to change the analysis settings.

10.   Click **Next**. The **Plate Layout** tab opens.

11.   Add standards, controls, unknowns, and maintenance steps to the plate layout.

12. Click **Save** to save the protocol.

Creating a Kit

To create a kit:

1. Open the **Protocols** page, then open the **Protocols** tab.
2. Select the protocol that you want to use for the kit, then click **New Std/Ctrl**. The **Std/Ctrl Details** tab opens.
3. Type the name of the kit in the **Name** box, the lot number in the **Std/Ctrl Kit Lot#** box, the expiration date using MM/DD/YYYY format in the **Expiration** box, and the manufacturer in the **Manufacturer** box.
4. Click **Apply Std Lot** if you want to apply a standard lot. The **Select Lot** dialog box opens. Select a lot and click **OK**.
5. Click **Apply Ctrl Lot** to apply a control lot. The **Select Lot** dialog box opens. Select a lot and click **OK**.
6. Alternatively, type the appropriate information in the **Assay Standard Information** and **Assay Control Information** sections. The number of standards, controls, or both in these sections is defined in the protocol. If your batch uses controls, select **Expected**, **Low** or **High** from the **Show Value** options. Use the **Apply Values** arrows to apply values down or across the range of analytes.
7. Click **Save**.

Creating a Batch

There are three batch creation options:

- **Create a New Batch from an Existing Protocol** (uses a protocol; described in this section)
- **Create a New Batch from a New Protocol**
- **Create a New Multi-batch** (runs multiple batches on a single plate at one time)

To create a new batch from an existing protocol:

1. Open the **Batches** page.
2. Click **Create a New Batch from an Existing Protocol**.
3. Type the batch name in the **Batch Name** box.
4. Type a description of the batch in the **Enter Optional Description** box.

5. Click the protocol you want to use in the **Select a Protocol** list. If the protocol you select uses standards and/or controls, the active reagents are shown on the bottom of the screen. Verify that these are the correct standards and controls.

6. Click **Next**.
   • If the protocol you selected uses standards and controls, the **Stds & Ctrls** tab opens. View the details of the active reagents and verify that they are correct; apply different assay standards and controls, or manually enter new information. Click **Next**. The **Plate Layout** tab opens. Confirm that the plate layout conforms to your specific assay instructions.
   • If the protocol you selected does not use standards and/or controls, the **Plate Layout** tab opens. Confirm that the plate layout conforms to your specific assay instructions. Continue with this step.

7. On the **Plate Layout** tab, add samples to the plate layout if needed.

8. Click **Run Batch** to begin batch acquisition, or click **Save** to save the batch information to the **Pending Batch** list, to be run at a later time.

   NOTE: If the batch spans more than one plate, the tray ejects automatically when all defined wells have been acquired. A dialog box prompts you to insert the next plate.

   NOTE: If running a Multi-batch, ensure the Plate Name type is the same when setting up the Batch protocol(s).

Viewing Batches

You can observe and analyze current and previously run batches on the **Results** page.

To view batch data:

1. Open the **Results** page.
2. Click the **Current Batch** or **Saved Batches** tab.
3. If you want to open a saved batch, highlight the batch and click **Open**.

Shutting Down the Instrument

Run the daily shutdown routine to prevent clogs and crystallization of salt in the sample probe. Clogs and crystallization of salt in the sample probe may cause problems with calibration, verification, and data acquisition; they may also cause sample splashing. Shut down the system properly to ensure system integrity.

1. On the **Home** page, click **Shutdown**. The **Auto Maint** tab opens, with **System Shutdown** selected.
2. Fill the labeled area of the off-plate reagent block (**RB2**) with a 10% to 20% household bleach and water solution.
3. Add deionized water to the labeled area of the off-plate reagent block (**RA1**).
4. Click **Run**.

Technical Support

For more information, visit the Luminex FAQ page at *http://www.luminexcorp.com*.

You can access the Technical Support website using a user name and password at *http://www.luminexcorp.com*.

Contact Luminex Technical support at:

- Phone: 512-381-4397
- Toll Free: 1-877-785-2323
- International: + 800-2939-4959
- Fax: 512-219-5114
- Email: *support@luminexcorp.com*.

   NOTE: When contacting Technical Support, have the instrument serial number available for reference. It is also recommended that you generate a Support Utility File (SUF) for submission to Technical Support. For information on sending a support file, see the "*Support Utility Tab*" section within the *Luminex® xPONENT® 4.2 Software User Manual IVD.*