# Luminex<sup>®</sup> xPONENT<sup>®</sup> 3.1 Quick Guide



This guide outlines the basic procedures for routine use of the Luminex xPONENT 3.1 system. For detailed instructions, refer to the appropriate Luminex Hardware User Manual or the Luminex xPONENT3.1 Software User Manual. © Luminex Corporation, 2001-2013, All rights reserved. No part of this publication may be reproduced, transmitted, transcribed, or translated into any language or computer language, in any form or by any means without prior express, written consent of:

LUMINEX CORPORATION

12212 Technology Boulevard



Austin, Texas 78727-6115

U.S.A.

Voice: (512) 219-8020

Fax: (512) 219-5195

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The Luminex 100/200 is a class 1(I) laser product.

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# **Open xPONENT 3.1**

When you turn on the computer attached to your Luminex system, xPONENT software opens automatically. If it does not open, click the Luminex icon on the desktop, or:

- 1. Click Start.
- 2. Click Programs.
- 3. Click Luminex.
- 4. Click **xPONENT**.
- 5. Click Luminex xPONENT.

The System Login page appears. Type your user name, and if required, your password. The **Home** page appears. You can access most tasks from the **Home** page.

### Start-up Indicators

When you turn on the instrument, the following start-up indicators occur:

- The blue light on the analyzer, located above the sample probe, turns on.
- The blue light on the XY Platform (XYP<sup>™</sup>) turns on.
- The blue light on the Sheath Delivery (SD<sup>™</sup>) turns on.
- The compressor engages and emits a rumbling sound.
- Air and sheath pressure build in the system.
- Air blows from the fans at the back of the analyzer and the XYP.
- The syringe pump initializes and emits a high-pitched sound as the syringe plunger makes a full stroke.
- As the unit primes, sheath fluid drips into the waste bottle.

### The System Monitor

The System Monitor, located at the bottom of the screen, displays continuous system status updates.



#### FIGURE 1. System Monitor

## Software Overview

xPONENT 3.1 software is organized into a series of pages, with a menu for each page. As you click each menu, applicable tabs appear on the left-hand side of the screen.

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

#### FIGURE 2. The Home Page.

Home	Samples	Batches	Results	Protocols	🥡 Mainte	mance 🛛 🚷 Admin
Home	Welcome, Admini Instructions R Click to 0 from a re	istrator un a daily activity, routine Create a new Batch ew Protocol	e, or select a protocol from	Nyour list to create a new batch Click to Create a new B using the highlighted Pr below	atch otocol	Daily Activities
	Installed Protocols		(1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.			Laser warm-up, fluidics, calibration performance verification
	Name	Version	Manufacturer	Date	Scroll	
	New Protocol 2	1		2(12)2008 4:43 PM	4	6 Shutdown
	New Protocol 2	5		3/13/2008 4:57 PM	View	Sanitze, wash, soak Probe and Heater Adjust the probe, set up the heater.
						<b>%)</b>
						Sys Info Reports
m Status Com	and:	Complete		Delta Cal Temp:	Laser:	
Syster	n Slate:			Sheath Pressure:	Region E	vents:

xPONENT 3.1 includes the following pages:

Home	Access most commonly used features
Samples	Import or enter sample data (optional)
Batches	Set up batches (experiments)
Results	Analyze previously acquired batches
Protocols	Set up protocols (called templates in IS 2.3) used to run batches
Maintenance	Calibrate, verify, and maintain the application
Admin	Perform administrative tasks
Log Off	Log out of software
Help	Access help file

# Adjust the Sample Probe Height

Adjust the sample probe height if:

- You change the type or style of plate being used.
- You remove and replace the sample probe for cleaning or troubleshooting.
- You move the instrument.
- You observe slow or sporadic sample acquisition.
  - **CAUTION:** Correct sample probe height is critical to successful sample acquisition and calibration. Problems with the sample probe can lead to fluid leaks and pressurization problems, as well as inhibit sample acquisition. To adjust the height of the sample probe, follow the steps for your system. For detailed instructions, see the Luminex LX100 or LX200 manual that you received with your instrument.

To adjust the sample probe height:

- 1. On the Home page, click Probe and Heater. The Probe & Heater tab opens.
- 2. If you are using a filter-bottom plate, place 3 large alignment disks in a well. For other v-bottom plates, use one sphere; for a round-bottom plate, use 2 alignment disks; for a flat-bottom plate, use 2 large alignment disks.
- **3.** Ensure that the well location is selected on the plate image, preferably A1 (a blue pin marks the location). To change the well location, click on a well in the plate image.
- 4. Verify that the microtiter plate is not warped. Warped plates can lead to incorrect probe height adjustment.
- 5. Click Eject to eject the plate holder.
- 6. Place the plate on the plate holder.
- 7. Click Retract to retract the plate holder.
- 8. Select the plate type from the Plate Type drop-down list.
- 9. Click Sample Probe Up to raise the sample probe.
- 10.Gently loosen the probe adjustment screw. Move the probe to its highest position.
- **11.**Click **Move Probe Down**. Loosen the probe adjustment screw and move the probe down until it makes contact with the alignment disc or sphere. Tighten the adjustment screw.
- 12.Click Sample Probe Up to raise the sample probe.
- **13.**Open the XY door. Click **Move Probe Down** and watch as the probe moves down. The black plate should not bounce when the probe reaches the down position.
- 14.Click Sample Probe Up.
- 15. Click Eject to eject the plate holder. Remove the alignment tools from the plate.

16.Replace the plastic shield that covers the sample probe area.

FIGURE 3. Move the Probe Up and Down

Auto Maint	Adjust Probe and Plate F Instructions Select ap	Heater plate location and adjust the probe	height. Turn the plate heater on or of	f, and adjust its temperature.	e
Lot Management	Adjust Probe Height	Plate Type: Automated M	aintenance 🗸	Plate Heater	
Cmds & Routines				ON OFF	
Probe & Heater		XMAD	DI H2O (87)		
System Info	Move Probe	TECHNOLOGY Automated Maintenance Plate		Set Temperature	
System Status	Down		70% ЕЮН (27)	Apply	
Schedule	( Land		10% Bleach (H7)	Current Temperature.	
Support Utility				25.6 ° C	
				40.0 ° C	

### Initialize the System

xPONENT 3.1 enables you to customize system initialization using one of these three options:

- Warmup and fluidics.
- Warmup, fluidics, and verification.
- Warmup, fluidics, verification, and calibration.

To run the system initialization routine:

- 1. On the Home page, click System Initialization.
- 2. Select the performance verification kit from the Performance Verification Kit list.
- 3. Select the calibration kit from the Calibration Kit list.
- **4.** Vortex the xMAP calibrator, verification, and fluidics containers to ensure homogeneity. *Do not dilute xMAP calibrator or verification agents.*
- 5. Click Eject on the status bar.
- 6. Place the Automated Maintenance Plate (AMP) on the plate holder.
- 7. Load the plate with at least five drops of the beads in the Calibration and Performance Verification Kit, as indicated on the plate image and in the kit insert instructions.
- 8. Click Retract.
- 9. Click Run.

**NOTE:** Perform a verification daily to check system integrity and to ensure that calibration is still valid.

#### **FIGURE 4. System Initialization Routine**

Auto Maint	Instructions	Select the Auton	nated Maintenance Op	tion or Maintenance	Command. Then, sele	ct the appropricate k	ot if applicable.	
Lot Management	System Initialization	Performance Verification	Celibration / Verification	System Shutdown	Alcohol Plush	Sarifize		
Cmds & Routines	Command	Location	Status	Information	Reagents			-
	Warmup		Pending		Calibration Kit		Performance Verification Kit	
Probe & Heater	Prime		Pending		AXXXXXX	*	A20000Y	
	Alcohol Flush	1,E7	Pending		Alcohol Flush	Vortex each reagent	vial for 30 seconds. Place 5 drops	ot
Sustam Info	Wash	1,87	Pending		Senitze	reservoirs with DI H2	0 and 70% isopropanol.	
oystem mit.	Wash	1,87	Pending		Wash Coak		10075120	Comm
	CAL1	1,A10	Pending		Drain	YAAA	B \$1 H2D (87)	
System Status	MagCAL1	1,810	Pending		CONT	TECHNOLO	T 🔘 🗐	
	CAL2	1,C10	Pending		MagCON1	Automated Basterana Park	000	the Sys
Schedule	CONT	1,A11	Pending		Gene CON2		70% EXOH (E7)	Initializ
	MagCON1	1,B11	Pending		CALI Marchill		000	Routin
Support Utility	CON2	1,C11	Pending		CAL2		000	
	Wash	1,87	Pending		Fluidics1		10% Blaach (HT)	2
	Fluidics1	1,D11	Pending		Fluidies2		000	
	Fluidics2	1E11	Pending		Multiple			_

### Calibrate the Instrument

Calibrate the instrument weekly. You should re-calibrate if:

- The delta calibration temperature exceeds ±3 degrees Celsius.
- You move the system.
- You perform maintenance on the system.
- System acquisition is low or sporadic.

To calibrate the system, click the **Maintenance** menu. The **Maintenance** page opens. Click the **Auto Maint** tab. Select the routine you want to execute and click **Run**.

### Create a Protocol

You can create four types of protocols using xPONENT 3.1:

- Quantitative Used to analyze unknown data generated with a standard curve.
- Qualitative Used to analyze unknown data generated with cutoff ranges based on one standard.
- Allele Call Used to analyze 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 3.1 reports are unavailable for data acquired with a protocol of type "none.")

You can create each quantitative, qualitative, and allele call protocol 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** menu, **Protocols** tab. The three steps are:

- 1. Define settings.
- 2. Select the analysis type.
- **3.** Define plate layout.

To create a protocol:

- 1. Open the Protocols page.
- 2. Click Create New Protocol. The Settings tab opens.

#### FIGURE 5. Settings Tab on Protocols Page

Protocols	Step 1: Pr Instructio	ns Name this	protocol and select the acqu	isition settings. Press	Next to continue.		
	Name	New Protocol 2		En	ter optional descrip	tion here	
Settings	Version	1	Manufacturer:				
2) Analytes		Acquisition Set	tings				
		Bead Type:	MicroPlex	~	DD Gating:	7500 to	13500
3) Plate Layout		Volume:	50 microliter	5	Reporter Gain:	Default	×
		Timeout	Enabled 200	esconde			
Stds & Ctris		XX Hostor	Enabled 200	doproue C			
		Analysis Settin	gs				
		Analysis Type:	None	Min MFI . Enabled	🖂 Ana	alyze results while acq	uiring samples
		Number	of Standards:		🗆 Use	e External Analysis Pr	ogram
		Numbe	r of Controls.		Analys	as Program	
		(*)	Fit of all Standards ု Mean	of Replicates			
_							

- 3. Type the protocol name, version, and manufacturer.
- 4. Type the following information in the Acquisition Settings panel:
  - Bead type (MicroPlex or MagPlex)
  - Sample volume (20-200µL)
  - Timeout (1-250 seconds)
  - XY heater temperature (35-60°C)
  - DD gate (buffer dependent)
  - Reporter gain (Default or High PMT)
- 5. Select 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.

	Stan 2: Salact	Analytan for "Naw Pro	atosol 2"					6.00
Protocols	Instructions	Select analytes. Edit ana Analysis column to set th	alyte name, units, counts, and s ne normalization bead.	elect an intra-well normalizat	on bead, if desired. Select a	in analyte or	n the	0
6	Analytes	1		Default Analysis:	Change	its: Cou	nt	Apply A
1) Settings	Select All		5 8 7 8 9 1	Tatal Coust	Stop after total head co	int reaches	100	
Analytes	Deselect All	11 12 12 14	15 16 17 18 19 2	Name	Analysis	L Inits	Count	Regio
		21 22 23 83	3 3 7 3 3 3	Analyte 23	No Analysis	Guild	100	23
3) Plate Layout		3 2 0 0	337338	Analyte 24	No Analysis		100	24
τ			45 46 47 48 49 5	Analyte 33	No Analysis		100	33
Stds & Ctris		6 6 6 4 (		Analyte 34	No Analysis		100	34
				•				
					Cancel	Ba		Next
stem Status Connec	flori: Connected			Delta Cal Temp:	Laser:			
stem Status	dion: Connected end:   State: Active			Delta Cal Temp: Sheath Pressure:	Laser: Region Event	s:	_	

#### FIGURE 6. Select Analytes

9. Click **Change** to change the analysis settings.

**10.**Click **Next**. The Plate Layout Tab displays.

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

12.Click Save to save the Protocol.

#### FIGURE 7. Plate Layout Tab



## Create a Kit

To calculate values for unknowns in your assay, enter specific values for standards and controls when you set up your qualitative or quantitative analysis protocol. Because standard and control values can change from lot to lot from the same manufacturer, enter these values separately from the protocol, as either lots or kits. If you initially enter standards and controls as kits and then save the lots individually, you can reuse the lots individually in later applications.

To create a kit:

- 1. On the Protocols page, click the Stds & Crtis tab.
- 2. Click Create New Std/Ctrl Lots. The Std/Ctrls Details page appears.
- 3. Select the appropriate protocol and click OK.
- 4. In the Lot Details panel, enter the following information:
  - Kit name, Std/Ctrl lot number, expiration date, and manufacturer
  - Assay standard or control information such as name, lot number expiration, manufacturer, and standard or control values for each analyte

**NOTE:** You can enter expected, high, or low values for control information. Click the appropriate button to make your selection.

Once kits and lots are created, they can be used with other protocols that use the same analytes.

#### FIGURE 8. Lot and Std/Ctrl Details

U U		•								•
rotocols	Lot and Std/Ct Instructions	Create or edit	a standard and	control lot. To	o group lot as S	Std/Ctrl Kit, a	iso fill out Std	/Ctrl Kit inform	nation.	
	Enter a kit name	to create a kit								
tds & Ctris	Apply Std/Ct	I Kit Name:	<u> </u>	Std/Ctrl Kit	Lot #	1	Expiration:		Manufacturer:	
Std/Ctrl Detail	Assay Standard	Information								
	Apply Std Lo	Show Qu	ualitative Fact	or	Apply Volues	*	4			
	Reagent Ne Standard1	rne Lot∦	Expirat	Manufa	Analyte 44	Analyte 45	Analyte 46	Analyte 54	Analyte 55	Analyte 56
	Reagent Ne Standard1 e: Assay Control II Apply Ctrl Lo	formation Shov	Expirat	Manufa /	Analyte 44 /	Analyte 45 Apply Vi	Analyte 46 alues:	Analyte 54	Analyte 55	Analyte 56
	Reagent Na Standard1	rne Lot# formation t Show © Ex mme Lot#	Expirat v Value pected O t Expirat	Manufa Low O H Manufa	Analyte 44 /	Analyte 45 Apply Vi Analyte 45	Analyte 46 alues Analyte 46	Analyte 54	Analyte 55 Analyte 55	Analyte 56 Analyte 56
	Reagent N Standard1 C Assay Control N Reagent N C	rne Lot# formation t Shov © Ex me Lot#	Expirat	Manufa Low O H Manufa	Analyte 44 /	Analyte 45 Apply Vi Analyte 45	Analyte 46 alues:	Analyte 54	Analyte 55 Analyte 55	Analyte 56 Analyte 56

### Create a Batch

You can run batches with or without protocols. 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 (does not use a protocol).
- Create a New Multi-batch (runs several batches).

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. 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.
- 5. Click Next.
  - If the protocol you selected uses standards and controls, the **Stds & Ctrls** tab appears. 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 appears. Go to step 6.
  - *If the protocol you selected does not use standards and/or controls*, the **Plate Layout** tab appears. Continue with step 6.
- 6. On the **Plate Layout** tab, assign well commands for this batch. Add samples to the plate layout if needed.
- 7. 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.
- **TIP:** Consider using the Single Step option when running batches. Most errors will be apparent in the first sample, and you can correct them without losing samples. You can observe real-time data collection on the **Results** page.

### View Batches

You can observe and analyze current and previously run batches on the **Results** page. Batches run with a quantitative, qualitative, or allele call protocol can be analyzed (in other words, if you selected "none" as your protocol, you will not be able to analyze data).

To view batch data:

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

#### **FIGURE 9. Viewing Current Batch Results**



## Run, Save, and Print Reports

xPONENT 3.1 can provide information in three different report types:

- Batch
- Protocol

• Advanced (User)

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

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

- 1. Click the **Results** page.
- 2. Click the **Reports** tab.
- 3. In the **Report** list, click the report you want to view.
- 4. In the Type list, select the type of report you want to view.
- 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. The report appears in the lower part of the Reports tab.
- 7. Click Print to print the report, or Save to save the report as a PDF file.

To view a data interpretation report:

- 1. Open the Results page.
- 2. Select the Reports tab.
- 3. Select New Report, then Batch Report, then Data Interpretation.
- 4. Select a previously run batch.
- 5. Click Generate Report.
- 6. Click Print to print the report, or Save to save the report as a PDF file.

#### FIGURE 10. Data Interpretation Report



### Shut Down the Instrument

Run the daily shutdown routine to prevent clogs and crystallization of salt in the sample probe. Shut down the system properly to ensure system integrity.

To shut down the instrument:

- 1. On the Home page, click Shutdown. The Auto Maint tab opens, with System Shutdown selected.
- 2. Fill the labelled area of the AMP with a 10-20% household bleach and water solution.
- 3. Add deionized water to the labelled area of the AMP.
- 4. Click Run.

# Appendix A: Common Daily Tasks

This flowchart illustrates the flow of common daily tasks. For more information about any task, see the hardware manual or xPONENT 3.1 Software User Manual.



# Appendix B: Technical Support

### On the Web

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

You can access the Technical Support website using a user name and password at <a href="http://www.luminexcorp.com/support">http://www.luminexcorp.com/support</a>

### By Phone

Inside the U.S. and Canada

Phone: 1-877-785-BEAD (-2323)

Fax: 512-219-0544

#### Outside the U.S. and Canada

Phone: +1 512-381-4397

### Email

Email questions to support@luminexcorp.com

### Luminex xPONENT 3.1 Software User Manual

For detailed information about the functions, features, and use of Luminex xPONENT 3.1, see the *Luminex* xPONENT 3.1 Software User Manual, available for download at <u>http://www.luminexcorp.com/support</u>