

LUNA II™

Automated Cell Counter

User Manual



DISCLAIMER

The contents of this document are subject to change without notice.
The LUNA-II™ Automated Cell Counter is an electrical laboratory instrument for scientific research use only.
It is not a medical, therapeutic, or in vitro diagnostics device.
Do not disassemble the device on any occasion as this will invalidate your warranty.

TRADEMARKS

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The WEEE (Waste Electrical and Electronic Equipment) symbol indicates that users of this instrument have the responsibility of returning and disposing of WEEE in an environmentally friendly manner. Follow the waste ordinances of your region for proper disposal provisions.



The CE mark indicates that this instrument conforms to all applicable European Community provisions for which this marking is required. Users must be aware of and follow the conditions described in this manual for operating the instrument. The protection provided by the instrument may be impaired if the instrument is used in a manner not specified by this manual.



Protective earth (Ground)

FCC COMPLIANCE

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at the user's own expense.

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Safety Precautions

Read this manual carefully before you begin to use this instrument to ensure that you know how to operate it safely and correctly. Use the instrument as specified by Logos Biosystems. Keep this manual in an easily accessible location for future reference.

1. Install the instrument on a sturdy and level surface. Avoid vibrations from other devices.
2. Do not touch any components with wet hands.
3. Operate the instrument in the conditions described in the Environmental Conditions for Operation.
4. Use the components provided or authorized by Logos Biosystems. If the proper combination of components are not used, product safety performance cannot be guaranteed.
5. Always use the power cord and AC adapter and provided by Logos Biosystems. If the proper power cord and AC adapter are not used, the electrical safety of the product cannot be guaranteed.
6. Ensure that the input voltage is compatible with the instrument's power supply voltage.
7. Ensure that the grounding terminal of the instrument and electrical outlet are properly connected. If the instrument is not grounded, the electrical safety of the product cannot be guaranteed.
8. Turn the instrument on only after connecting the power cord and AC adapter to both the power source and the instrument. Turn the instrument off before disconnecting the power cord and/or moving the instrument.
9. Disconnect the power cord after operation or in the case of abnormalities.
10. Do not disassemble the instrument in any event. If the instrument is malfunctioning or broken, please contact your local distributor or Logos Biosystems. Disassembling the instrument invalidates its warranty.
11. When connecting the USB drive to a computer, be careful not to be infected by computer viruses.
12. When disposing of this instrument, check and observe the rules and regulations of your local government.
13. Wear proper personal protective equipment (PPE) when handling stains and cell samples to avoid exposure.
14. Do not reuse LUNA™ Cell Counting Slides. Used slides must be disposed as biohazardous waste according to the rules and regulations of your local government.
15. The LUNA-II™ Automated Cell Counter is an electrical laboratory instrument for scientific research use only. It is not a medical, therapeutic, or in vitro diagnostics device.

General Guidelines

Follow the instructions below to obtain the best results with the LUNA-II™ Automated Cell Counter.

1. Hold slides by the edges to avoid touching the optical surface. Take care that the optical surfaces of the slide do not become smudged, damaged, or contaminated.
2. When staining cells with trypan blue, perform cell counting within three minutes of mixing samples for accurate cell viability measurements. If necessary, count your sample twice (duplicate readings) and take an average. Otherwise, use Erythrosin B for an alternative that is less toxic to your cells.
3. As the LUNA-II™ is calibrated before shipping, recalibration before use is not necessary. See Section 2.3.5: Settings: Background Calibration to see when background calibration is necessary.

Environmental Conditions for Operation

Operating Power	100 - 240 VAC, 1.2 A
Electrical Input	12 VDC, 3.3 A
Frequency	50/60 Hz
Installation Site	Indoor use only
Operating Temperature	10 - 35°C
Maximum Relative Humidity	20 - 80%
Altitude	≤ 2,000 m
Pollution Degree	2

Chapter 1 – Introduction

1.1 Product Overview

The LUNA-II™ Automated Cell Counter is an image-based cell counting device that features an innovative autofocus liquid lens and a proven counting algorithm, providing a fully automated solution for cell counting and viability analysis. Simply prepare a cell sample solution and the LUNA-II™ does the rest, doing away with the subjectivity and time expenditure of manual cell counting.

The LUNA-II™ counting algorithm declusters clumpy cells and counts them individually with precision. Counted cells can be gated for size and sorted into a cluster map with a user-friendly, interactive software interface.

The LUNA-II™ provides:

- the total number of cells per mL,
- the number of live and dead cells per mL,
- the viability of cells (% live cells to total cells),
- cell images (optional: labeling live and dead cells as green and red circles, respectively),
- cell cluster maps (% of single cells, doublets, and triplets), and
- histograms of cell size distributions.

The LUNA-II™ automatically saves results as CSV files and provides the option to generate comprehensive PDF reports with the date, time, protocol used, cell images, and relevant histograms. The LUNA-II™ also provides the option to review previous data.

Both reusable and disposable slides can be used with the LUNA-II™. The LUNA™ Reusable Slide is compatible with the LUNA™ family of automated cell counters for both brightfield and fluorescence cell counting. Designed for cost-efficient and accurate cell counting, the LUNA™ Reusable Slide combines the economy of manual cell counting with the speed, accuracy, and convenience of automated cell counting. The disposable LUNA™ Cell Counting Slides maintain the highest standard of cell counting accuracy and offer the ultimate counting experience with no mess or cleanup.

1.2 Key Features

Key Features	Description
Compact, space-efficient design	Lightweight and compact, the LUNA-II™ maximizes space and may be used on a laboratory bench or in a biosafety cabinet.
Accuracy & precision	Sophisticated optical components and a proven counting algorithm provide accurate and reproducible results.
Autofocusing	A non-mechanical liquid lens efficiently and reliably autofocuses, removing human error and enabling accurate cell counting.
Easy-to-operate user interface	A straightforward and intuitive software allows users to capture and analyze cell count and viability data with ease.
Shortest time-to-results	With manual focusing, you are 10 seconds away from your data. With autofocus, a mere 15 seconds.
Built-in printer	An integrated thermal printer makes record keeping effortless.
Cell size & concentration range	Cells 3 to 60 µm in size at concentrations ranging from 5×10^4 to 1×10^7 cells/mL are easily analyzed.
Simple dilution calculations	Onboard software calculates dilutions for users.
Onboard memory	Up to 1000 counts can be saved directly to the LUNA-II™.
Customizable protocols	Up to 300 unique protocols can be set and used.
Data reports	Detailed PDF files complete with cell count and viability data, images, and histograms can be saved to an external drive.

1.3 Product Contents

The LUNA-II™ product package contains the following components.

Component	Quantity
LUNA-II™ Automated Cell Counter*	1
Power Cord (with AC adapter)	1
LUNA™ Cell Counting Slides, 50 Slides (100 Counts)	1 box
USB Drive, 16 GB	1
Trypan Blue Stain, 0.4%	2 x 1 mL

*The LUNA-II™ Automated Cell Counter with built-in printer (L40001) comes with one pack of LUNA-II™ Printer Paper installed.

Upon receiving the product package, please inspect its contents to ensure that all parts have been included and that no damage has occurred during shipping. The warranty does not cover damage that may occur during shipping and handling. Any damage claims must be filed with the carrier. Contact your local distributor or Logos Biosystems if anything is missing.

1.4 Product Specifications

LUNA-II™ Automated Cell Counter Specifications	
Instrument Type	Benchtop cell counter
Dimensions (W x D x H)	16 x 18 x 28 cm (6.3 x 7.0 x 11.0 in)
Weight	1.6 kg (3.5 lb)
Cell Concentration Range	5 x 10 ⁴ - 1 x 10 ⁷ cells/mL
Cell Diameter Range	3 - 60 µm (optimal range: 8-30 µm)
Cell Viability Range	0 - 100%
Image Resolution	5 MP
Image Type	TIFF
Processing Time*	10** (manual focusing) or 15** (autofocusing) seconds at ~1 x 10 ⁶ cell/mL

*Processing time may vary according to cell type and concentration.

**This is the minimum processing time for each focusing option at the specified concentration of HeLa or HL-60 cells.

LUNA™ Cell Counting Slide Specifications	
Material	Polystyrene
Dimensions (W x D x H)	25 x 75 x 2.4 mm
Chamber Depth	100 µm
Chamber Volume	10 µL

1.5 Product Description

1.5.1 Front and Right Side View of the LUNA-II™

The front of the LUNA-II™ has a wide touchscreen, a power button, a counting slide port to insert LUNA™ Cell Counting Slides or the LUNA™ Reusable Slide, and a USB port for easy data transfer. The right side of the LUNA-II™ has a built-in printer (L40001 models only), allowing the user to print results immediately.



1.5.2 Rear View of the LUNA-II™

The rear of the LUNA-II™ has two additional USB ports and a power inlet to connect the instrument to an electrical outlet.



Chapter 2 – Setting up

2.1 Installation

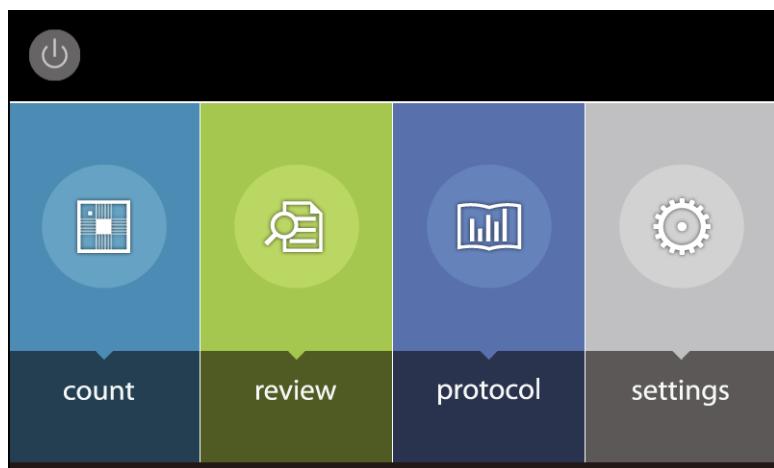
Place the LUNA-II™ on a clean and level surface. Connect the power cord to the AC adapter. Connect the power cord to an electrical outlet after checking the outlet configuration in your local area.

Do not install the instrument in a location that will expose the instrument to intense ultraviolet light.

2.2 Startup/Main Menu

Push the power button below the touchscreen to turn the instrument on. The company logo will appear, followed by the startup screen.

The main menu has a power icon and four options: **count**, **review**, **protocol**, and **settings**.



For instructions on when and how to turn the instrument on or off, see Section 6.1: Turning On/Off.

2.3 Settings

The instrument is preset at the time of manufacture and may be used immediately. Users may adjust the settings of the instrument as desired.

Select **settings** from the main menu.

The Settings screen displays:

- a home icon: press this icon to return to the main menu,
- the current protocol and date,
- the date and values of the most recent calibration, and
- the date and version of the latest software update.

Settings		Protocol DEFAULT	Date 16 Apr., 2017 13:12
Staining Options		Counting Options	Date / Time
	Software Update	Last Update	12 Apr., 2017 13:08
		Software Version	1.8.1
	Background Calibration	Last Calibration	12 Apr., 2017 14:58
		Calibrated Value	0x0469, 0x0BC1, 2200

Settings options:

- **2.3.1 [Staining Options]** Select for the presence or absence of a stain.
- **2.3.2 [Counting Options]** Select Auto Exposure mode to adjust light settings for each count.
- **2.3.3 [Date/Time]** Adjust the date and time of your instrument for record keeping purposes.
- **2.3.4 [Software Updates]** Update software to the most recent version.
- **2.3.5 [Background Calibration]** Perform background calibrations with each software update.

2.3.1 Settings: Staining Options

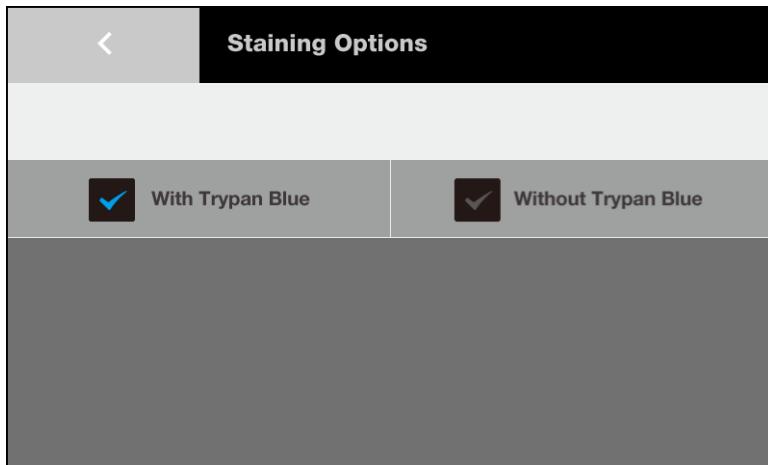
Users can select for the use or absence of a stain for cell counting with the LUNA-II™. The LUNA-II™ is optimized for use with trypan blue or Erythrosin B.

Option	Description
With Trypan Blue	This option is used when cell samples are mixed with trypan blue or Erythrosin B for regular bright field counting. This option generates cell viability data. Adjust the dilution factor in your set protocol appropriately.*
Without Trypan Blue**	When samples are not mixed with a stain, select this option and follow the directions in the message boxes. Adjust the dilution factor in your set protocol appropriately. *

*The dilution factor will not change automatically. Failure to adjust the dilution factor will lead to inaccurate cell concentration calculations.
**Low contrast from not using a stain may lead to suboptimal results.

Press **[Staining Options]** in the Settings screen.

The selected option will be marked with a blue .



Change the staining option by pressing the unselected option.

Select **With Trypan Blue** when using a stain such as trypan blue or Erythrosin B.

! **Important!** Users must recalibrate the background when switching stains (see Section 2.3.5: Settings: Background Calibration).

Press **OK** to select the desired staining option. Otherwise, press **Cancel** to close the window.

Press **<** to return to the Settings screen.

Choose the appropriate protocol or adjust the dilution factor accordingly (see Section 3.1: Protocol Parameters and Section 3.2: Creating and Editing Protocols).

2.3.3 Settings: Counting Options

Users can choose to turn Auto Exposure mode on or off. Auto Exposure mode automatically adjusts the light settings for each count to ensure the accuracy of counting results regardless of the brand, concentration, and shade of trypan blue used. Auto Exposure mode is especially important when using higher concentrations of trypan blue.

Press **[Counting Options]** in the Settings screen.

Select ON to activate Auto Exposure mode. Select OFF to deactivate Auto Exposure mode.

The selected option will be highlighted in blue.

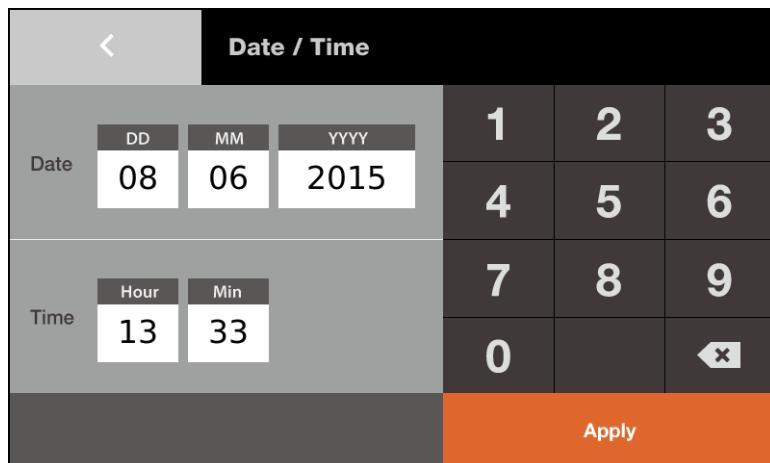
Press **<** to return to the Settings screen.

2.3.3 Settings: Date/Time

The LUNA-II™ uses a 24-hour clock and is preset to Korean time. Adjust the settings to the local date and time for accurate record keeping.

Press **[Date/Time]** in the Settings screen.

Select the desired field to delete the existing value.



Input the desired values with the number panel on the right. Press **Apply** to save changes.

Press **<** to return to the Settings screen.

2.3.4 Settings: Software Update

Logos Biosystems continually provides software updates to ensure optimal performance. The existing version of software is displayed in the startup screen and the Settings screen.

Download the most recent version from the Logos Biosystems website (www.logosbio.com) into the root directory of a compatible USB drive.

Press **[Software Update]** in the Settings screen. Insert the USB drive with the downloaded file into the USB port. Press **Start**. Do not turn the instrument off during the update. The date and version of the last software update will change automatically in the Settings screen.

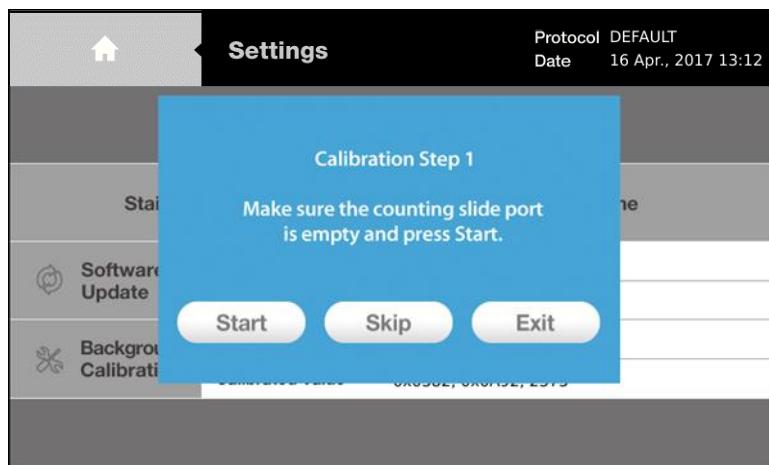
! **Important!** Users must recalibrate the background after each software update (see Section 2.3.5: Settings: Background Calibration).

2.3.5 Settings: Background Calibration

Background calibration adjusts for the specific shade of the stain used for counting and is a prerequisite for the successful detection of cells. Users must recalibrate the background after each software update or when using a different brand, concentration, or type of stain. The LUNA-II™ is optimized for use with trypan blue or Erythrosin B.

Press **[Background Calibration]** in the Settings screen.

A window will appear with directions for Calibration Step 1.



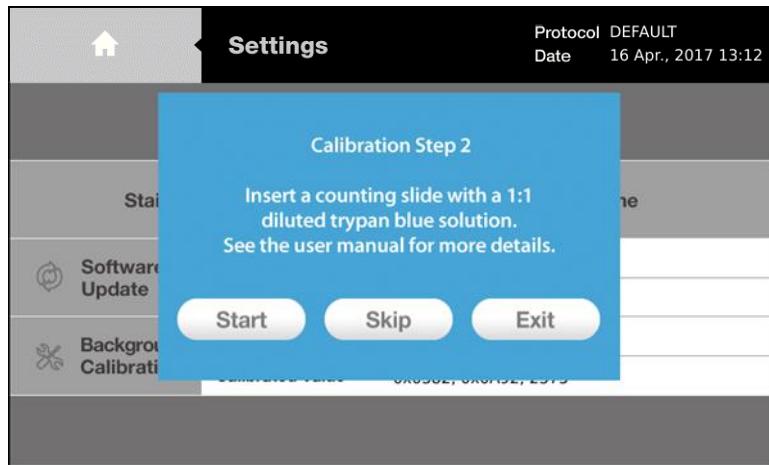
The counting slide port should be empty for Calibration Step 1. If there is a slide in the counting slide port, remove it from the instrument.

Press **Start**.

Do not turn the instrument off during this process.

Mix one part stain with an equal volume of distilled water, PBS, or plain medium. Put 10 μ L of the diluted stain into the chamber of a new LUNA™ Cell Counting Slide or a clean LUNA™ Reusable Slide.

A window will appear with directions for Calibration Step 2 when Calibration Step 1 is complete.



Insert the slide face up and sample-side first into the counting slide port.

! Important! Do not insert the slide facedown.

Press **Start**. Do not remove the slide or turn off the instrument during this process.

Press **Exit** to return to the Settings screen when Calibration Step 2 is complete. The background calibration value and date will have changed in the Settings Screen.

Chapter 3 – Protocol Settings

The LUNA-II™ provides a default protocol that can be used for most common cell lines. Users may create and save up to 300 unique protocols.

3.1 Protocol Parameters

The LUNA-II™ protocols have the following modifiable parameters:

Parameter	Range	DEFAULT	PBMC
Dilution Factor	1-100	2	2
Noise Reduction	1-10	5	5
Live Cell Sensitivity	1-9	1	7
Roundness (%)	0-100	60	60
Min. Cell Size (µm)	3-59	3	3
Max. Cell Size (µm)	4-60	60	30
Declustering Level	None, Low, Medium, High	Medium	Medium

Dilution Factor: The dilution factor is used to calculate cell concentrations accurately. The default dilution factor is preset as 1 for **Without Trypan Blue** and 2 for **With Trypan Blue**, assuming a 1:1 ratio of stain to cell suspension. Users can modify this value according to the dilution of the original sample in increments of 1 between 1-10 and of 10 between 10-100. For users handling highly dense cells (e.g. fermented CHO cells), serial dilutions and several counts with appropriately adjusted dilution factors will be necessary.

Noise Reduction: This option allows for the adjustment of background noise during counting. With more noise reduction, the instrument will be less sensitive and not detect weakly stained cells. With lower noise reduction, the instrument can detect objects with faint signals. Adjusting this parameter will help optimize for different cell types as staining can vary from cell to cell.

Live Cell Sensitivity: Live cells with intact cell membranes exclude trypan blue and Erythrosin B. The dyes form halos around live cells and stain the cytoplasm of dead cells or cells with compromised membranes. With a higher live cell sensitivity, the instrument can detect smaller cells by registering smaller halos. For most cells, this option should be set to 1. For peripheral blood mononuclear cells (PBMCs) or smaller, set it to ≥ 5 .

Roundness: Cells are not all completely spherical. Adjusting the roundness to detect cells of various shapes. Higher percentages lead to the counting of rounder cells and excludes objects with less roundness. Lower percentages are suitable for counting cells with irregular shapes.

Minimum and Maximum Cell Size: Users can customize cell size parameters to detect specific cells efficiently. Values can be adjusted in 1 µm increments for sizes between 3-60 µm.

Declustering Level: The declustering function allows for the efficient detection of a variety of cells that may clump or grow in clusters. Higher levels of declustering will increase counting time. This function is helpful for counting sticky cells or rod-shaped spores.

3.2 Creating and Editing Protocols

Select **protocol** from the main menu.

The Protocol screen includes a list of saved protocols. The selected protocol is highlighted in blue. The parameters of the selected protocol are displayed in the right panel.

Protocol		Protocol DEFAULT Date 16 Apr., 2017 13:12						
Protocol		Dilution Factor (1-100)	Noise Reduction (1-10)	Live Cell Sensitivity (1-9)	Roundness (0-100%)	Min. Cell Size (3-59µm)	Max. Cell Size (4-60µm)	Declustering Level
DEFAULT		▲	▲	▲	▲	▲	▲	▲
PBMC		2	5	1	60	3	60	Medium
New Protocol		▼	▼	▼	▼	▼	▼	▼
		Load	Edit		Delete		Save as	

The DEFAULT and PBMC protocols cannot be modified or deleted.

To create a new protocol, select **New Protocol** and press **Load**.

Press **Delete** to delete the selected protocol.

Press **Edit** to modify the selected protocol. This will activate the arrows for each parameter, turning them a solid grey. Press the arrows to adjust the values of each parameter as desired.

Protocol		Protocol DEFAULT Date 16 Apr., 2017 13:12						
Protocol		Dilution Factor (1-100)	Noise Reduction (1-10)	Live Cell Sensitivity (1-9)	Roundness (0-100%)	Min. Cell Size (3-59µm)	Max. Cell Size (4-60µm)	Declustering Level
DEFAULT		▲	▲	▲	▲	▲	▲	▲
PBMC		▲	▲	▲	▲	▲	▲	▲
New Protocol		▲	▲	▲	▲	▲	▲	▲
		2	5	1	60	3	60	Medium
		▼	▼	▼	▼	▼	▼	▼
		Load	Edit		Delete		Save as	

Press **Save as**.

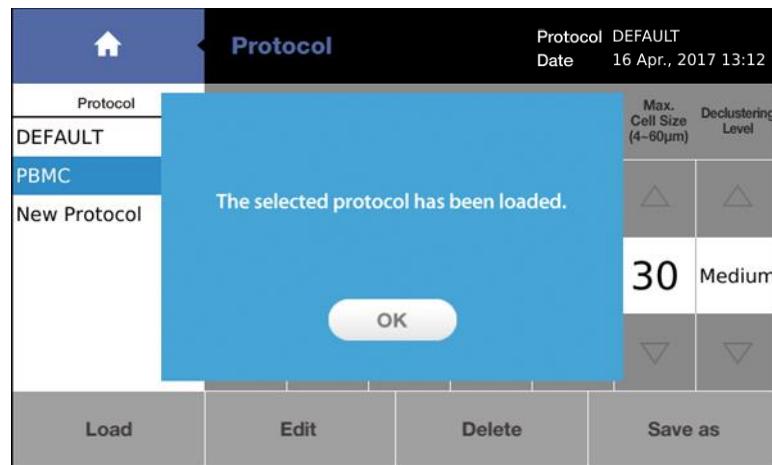
Using the onscreen keyboard, name the protocol and press **Save**.

The newly created protocol will appear in the list of protocols in the Protocol screen.

3.3 Protocol Selection

Select the desired protocol in the Protocol screen.

Press **Load** to apply the selected protocol.



Now the instrument is ready to count cells with the selected protocol.

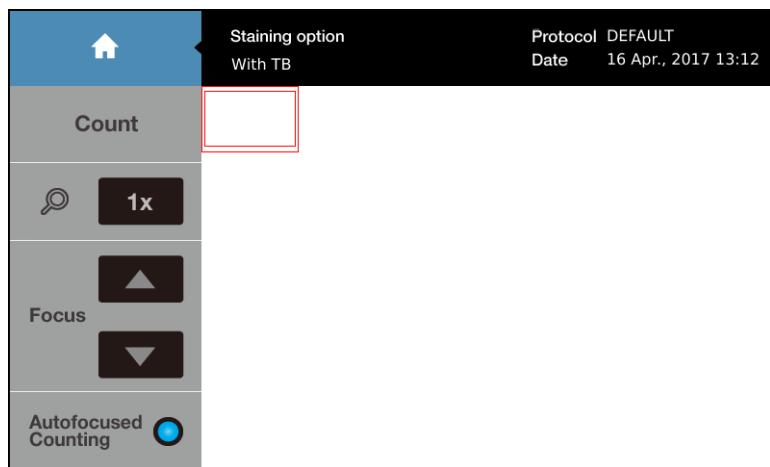
! **Important!** Merely selecting a protocol does not mean that it has been put into effect. To apply the selected protocol, make sure to press **Load**.

Chapter 4 – Counting Cells

4.1 Instrument Preparation

Select **count** from the main menu.

The staining option, set protocol, date, and time appear in the panel at the top of the Count screen.



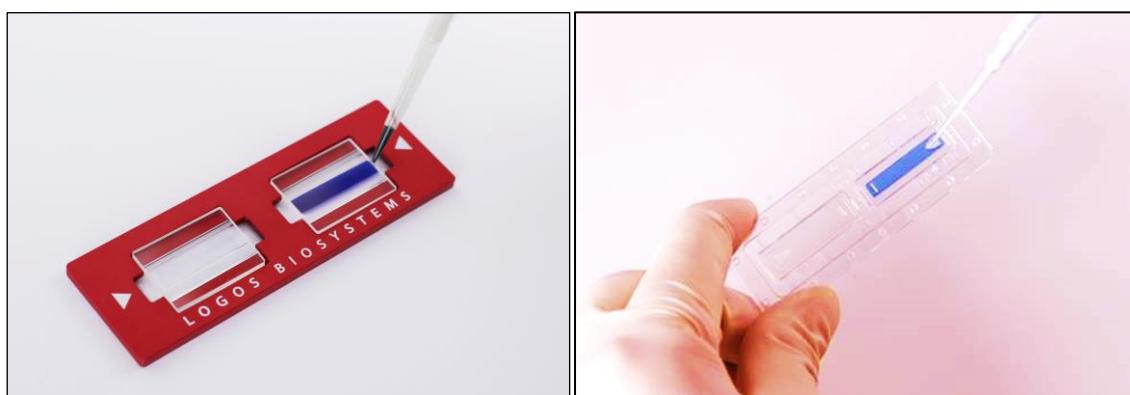
Set the protocol, dilution factor, and staining option appropriately prior to counting.

4.2 Sample Preparation

Prepare a cell suspension according to standard procedures. Mix gently but thoroughly to ensure that the suspension is homogenous.

Mix 10 μ L cell suspension with 10 μ L stain. Pipette gently.

Prepare a new LUNA™ Cell Counting Slide or a clean LUNA™ Reusable Slide. Hold the slide by its edges and load 10-12 μ L of the cell sample into a sample chamber. For easy and accurate loading, hold the pipette at a 45-60° angle to the slide. Be careful not to over-load or under-load the sample chamber.



4.3 Slide Insertion

Insert the slide face up and sample-side first into the counting slide port of LUNA-II™. The LUNA-II™ can only analyze the inserted chamber.

! Important! Do not insert the slide facedown.

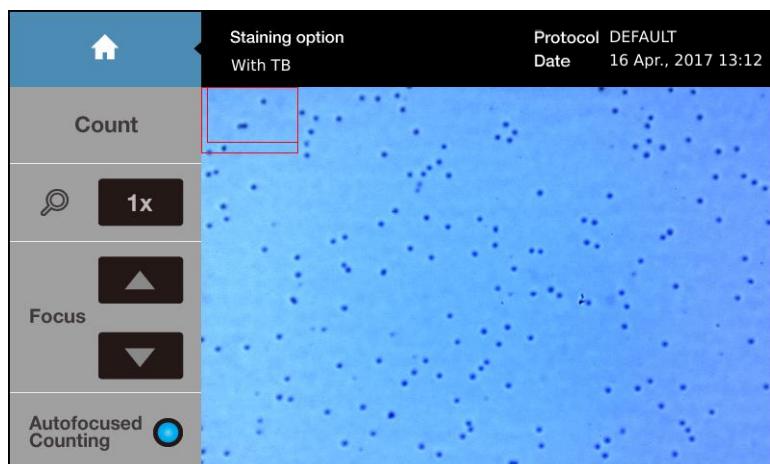
A live image of the cells will appear on the screen. If not, the slide might not be inserted correctly.

4.4 Focusing

The LUNA-II™ has an autofocus algorithm that works in tandem with a focusing mechanism to rapidly obtain the Z position of the sample by applying a small voltage to a liquid lens. The elimination of mechanical parts in the focusing mechanism removes noise and significantly reduces the need for servicing.

4.4.1 Autofocusing

Press the circle next to **[Autofocused Counting]**. The circle will turn blue when the autofocus is activated.

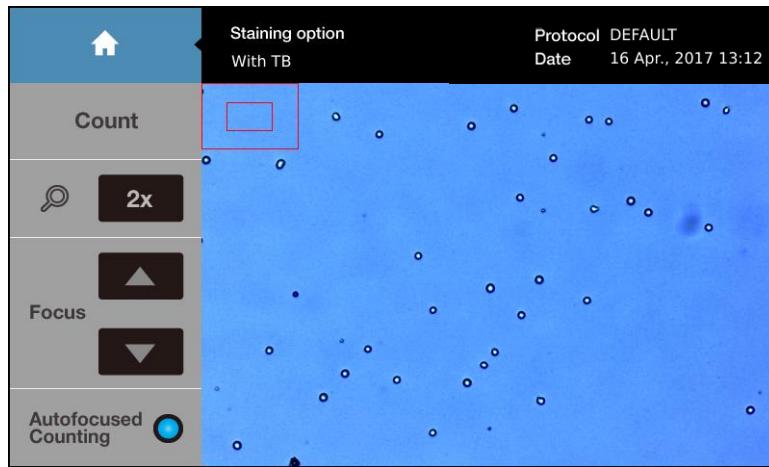


4.4.2 Manual Focusing

Users can adjust the focus manually by simply pressing the **[Focus]** arrow heads (up or down) with the autofocus function on or off.

4.5 Cell Counting

Use a finger or a stylus to navigate the image. The red outer box in the top left corner of the image represents the entire counting area and the inner box is the current field of view. The size and location of the inner box will change with the magnification and movement of the screen



Press the magnifier button to zoom in and out of the image.

Press **[Count]** to start counting.

The LUNA-II™ counts the cells in 0.5 μ L, which is comparable to five (1 mm x 1 mm) squares on a standard hemocytometer.

Counting time will vary with protocol, cell size, and cell concentration. With the DEFAULT protocol, cell samples with a concentration of $\sim 1 \times 10^6$ cell/mL will take at minimum 10 seconds to count without autofocusing or 15 seconds with autofocusing.

Cell count and viability results will appear.

Results		Protocol DEFAULT Date 16 Apr., 2017 13:12
Next Count	Total cell concentration	1.06×10^6 cells/mL
Image	Live cell concentration	9.18×10^5 cells/mL
Histogram & Gating	Dead cell concentration	1.38×10^5 cells/mL
Dilution	Viability	87.0 %
Save/Print	Average size	13.0 μ m
	Total cell number	230 cells
	Live cell number	200 cells
	Dead cell number	30 cells
	Dilution factor	2

4.6 Results

The LUNA-II™ has onboard data analysis software that allows users to analyze cell count and viability data immediately.

4.6.1 Results: Image View

Press **[Image]** to view the captured image of the analyzed cell sample.

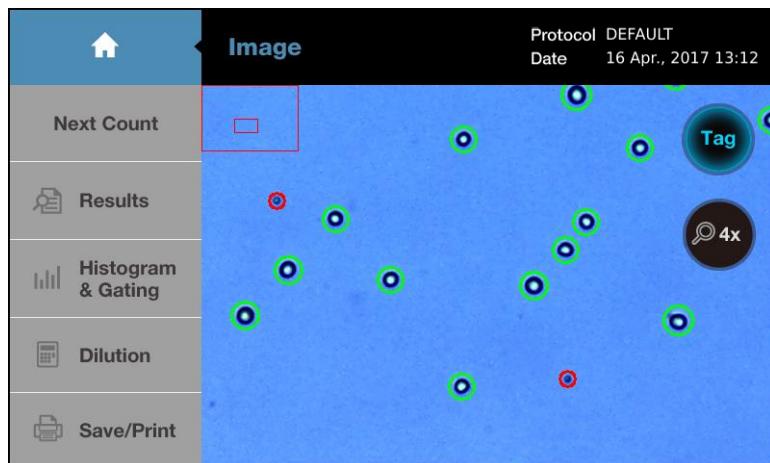
Results		Protocol DEFAULT Date 08 Jun., 2015 13:39
Next Count		
Image		Total cell concentration 9.18x10e5 cells/mL
Histogram & Gating		Live cell concentration 1.38x10e5 cells/mL
Dilution		Dead cell concentration 87.0 %
Save/Print		Average size 13.0 um
		Total cell number 230 cells
		Live cell number 200 cells
		Dead cell number 30 cells
		Dilution factor 2

Use a finger or a stylus to navigate the image.

The **Tag** and magnifier buttons are to the right of the image.

Press the magnifier button to zoom in and out of the saved image.

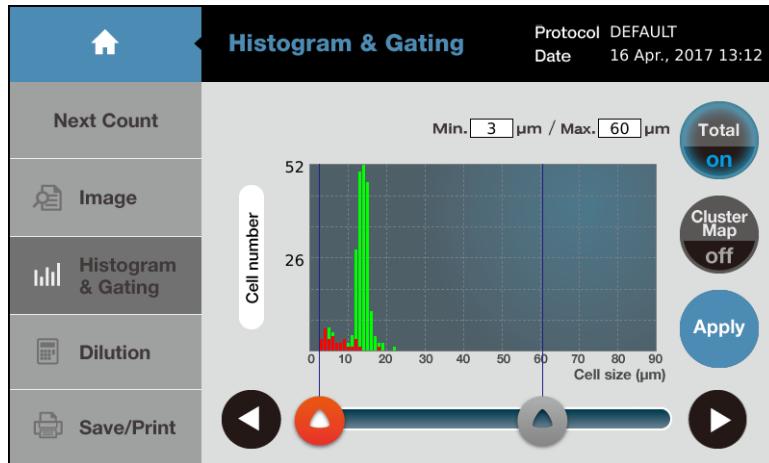
Press **Tag** to label what was counted as live cells with green circles and dead cells with red circles. The Tag function allows users to verify the instrument's counting accuracy immediately.



Press **Tag** again to remove the labels.

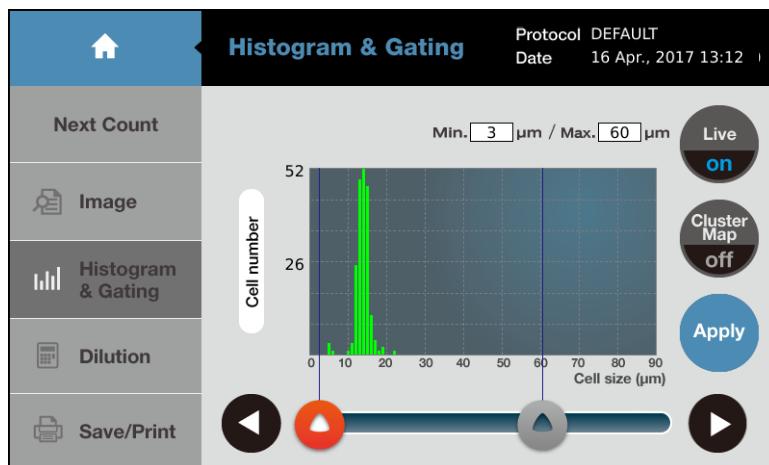
4.6.2 Results: Histogram and Gating

Press [Histogram & Gating] to see a graphical representation of the cell count results.

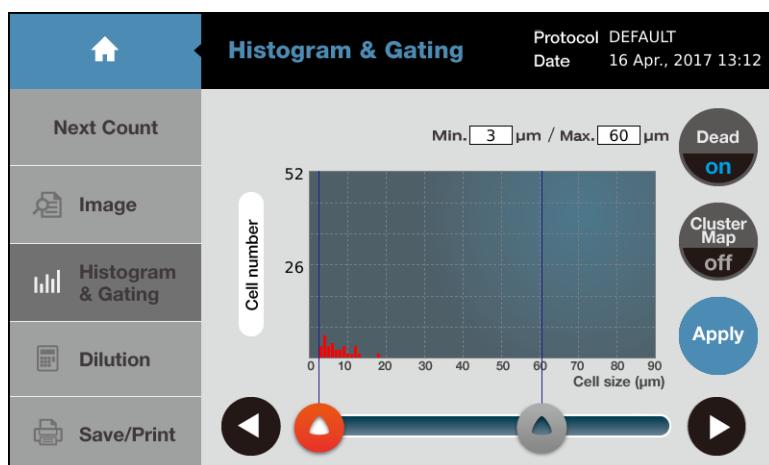


Users can review the distribution of cells according to their sizes. Green bars represent live cells and red bars represent dead cells. The **Total/on** button indicates that live and dead cells are both represented.

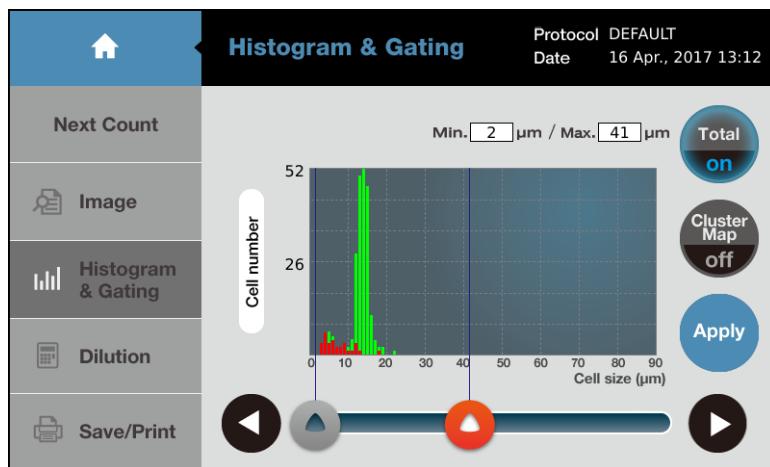
Press **Total/on** to change it to **Live/on** and display the size distribution of only live cells.



Press **Live/on** to change it to **Dead/on** and display the size distribution of only dead cells.



The LUNA-II™ provides a gating function that can be controlled by the gating bar on the bottom of the screen. Select the desired light grey limit icon. The selected icon will become red.

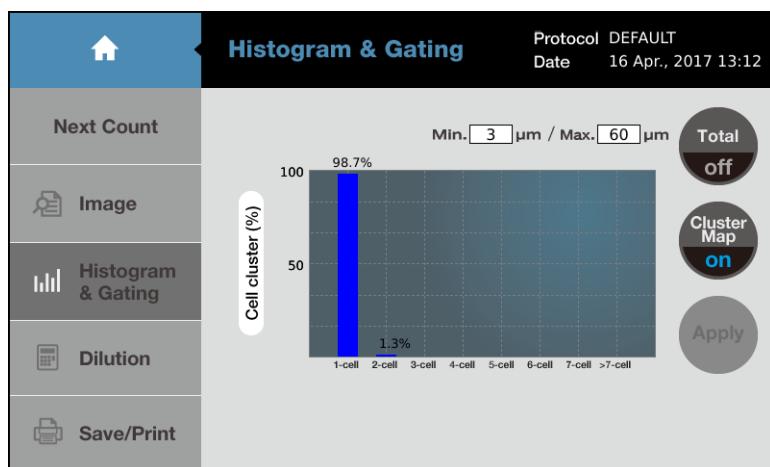


Press the arrows on either end of the size to alter the minimum and maximum size limits. The gating function is helpful for monitoring co-cultured cells with distinct sizes and the exclusion of noncellular particles.

Press **Apply** to set the size gating limits. The count results will adjust accordingly.

Press **Cell Number** to change the Y-axis to **Cell Concentration**.

Press **Cluster Map/off** to change it to **Cluster Map/on** and show the distribution of cell clusters.

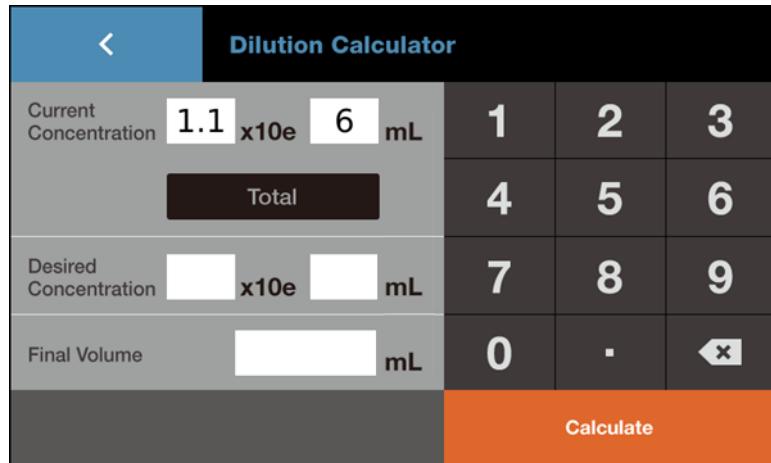


4.6.3 Results: Dilution Calculator

Users may use the onboard dilution calculator to compute dilutions for subsequent experiments.

Press **[Dilution]** and the dilution calculator will appear.

The dilution calculator starts out with the concentration of total cells (live and dead) as the current concentration. The current concentration options are **Total**, **Live**, **Dead**, and **Custom**, allowing users to set the current concentration to be the total cell concentration, live cell concentration, dead cell concentration, or a custom cell concentration by pressing the black box below the Current Concentration value.



The image shows the Dilution Calculator interface. It has a header 'Dilution Calculator' with a back arrow. The 'Current Concentration' field contains '1.1 x10e 6 mL'. Below it is a numeric keypad with digits 1-9, a decimal point, and a clear button. A 'Total' button is in the center of the keypad. The 'Desired Concentration' field is empty. The 'Final Volume' field contains '0 mL' and has a clear button. An orange 'Calculate' button is at the bottom right.

Input the values into the blanks for the desired final concentration and volume.

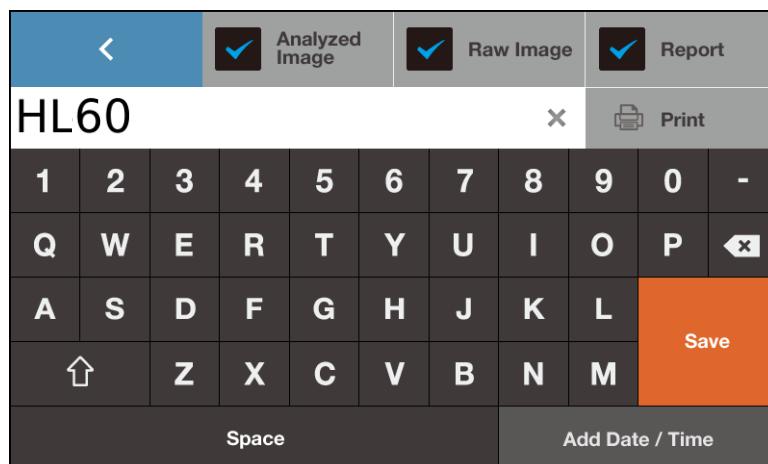
Press **Calculate**.

4.6.4 Results: Saving and Printing

The LUNA-II™ provides the option of saving and/or printing results.

Press **[Save/Print]** in the Results screen.

The Save/Print screen has three saving options.



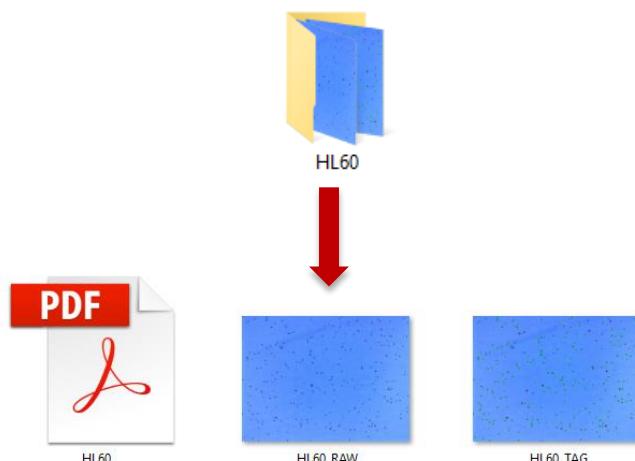
Saving Options	Description
Analyzed Image	Tagged image of live and dead cells
Raw Image	Untagged image of cells
Report	PDF report with count data and histograms

Select the desired saving options. The selected options will be marked with a blue .

Using the onscreen keyboard, name the count as desired.

Users may add the date to the name by pressing the **Add Date/Time** button.

Press **Save** to save to a USB drive. A folder of the same name will be created to contain all the files generated.



HL60.pdf

Cell Count Report

① ②

• File name: hl60 • Date: 16 Apr., 2017 14:35

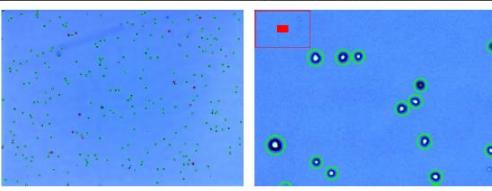
• Cell count results

Total cell concentration: 1.04×10^4 cells/mL
 Live cell concentration: 9.73×10^5 cells/mL
 Dead cell concentration: 6.62×10^4 cells/mL
 Viability: 93.6 %
 Average cell size: 16.5 μ m
 Total cell number: 251
 Live cell number: 235
 Dead cell number: 16

• Protocol

Protocol name: DEFAULT
 Dilution factor: 2
 Min. cell size: 3 μ m
 Max. cell size: 60 μ m
 Size gating: 3 ~ 60 μ m
 Noise reduction: 5
 Live cell sensitivity: 1
 Roundness: 60 %
 Declustering level: Medium
 Focusing method: Autofocus
 Staining option: With TB
 Counting option: Auto exposure(0x0330)

Cell Images (Average intensity: 148)



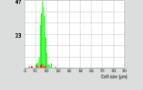
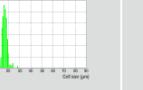
S/N: LUC-14-00000
 S/W version: 1.8.1

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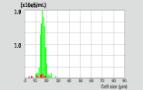
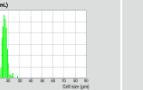
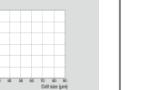
Cell Count Report

① ②

• Cell size distribution by cell number

	Total Cells	Live Cells	Dead Cells
Cell size (μ m)			

• Cell size distribution by cell concentration

	Total Cells	Live Cells	Dead Cells
Cell size (μ m)			

• Cell cluster map

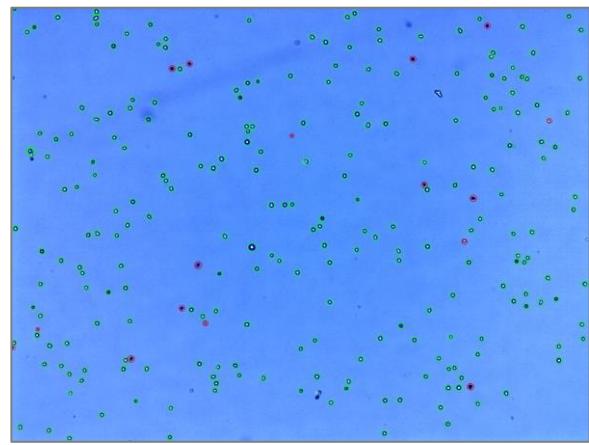
S/N: LUC-14-00000
 S/W version: 1.8.1

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HL60_RAW.tif



HL60_TAG.tif

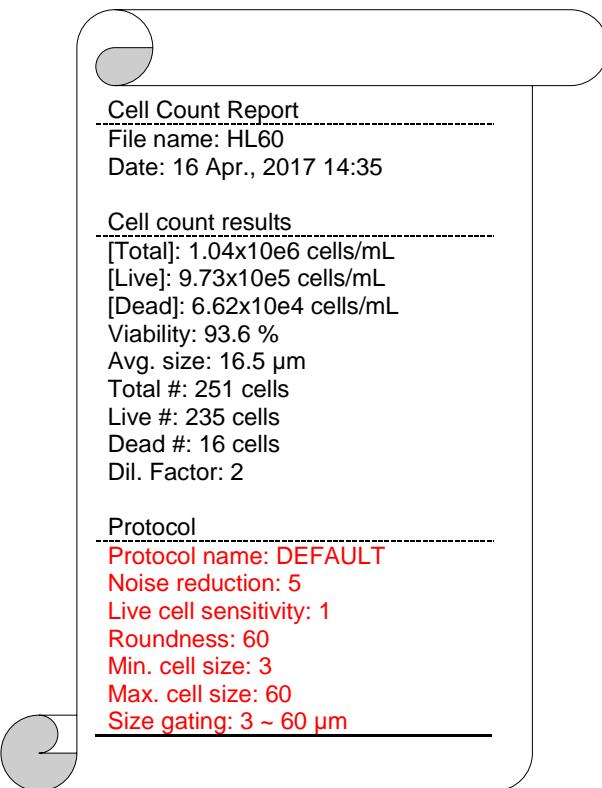


A summary of each count performed is automatically saved to the LUNA-II™.

The LUNA-II™ stores up to 1000 counts onboard.

Alternatively, press **Print**.

The printed report will contain the cell count results and protocol details.



Chapter 5 – Review Previous Results

The LUNA-II™ allows users to review previous results.

Select **review** from the main menu.

The review screen has two options: **[Review Files]** and **[Previous Counts]**. **[Review Files]** brings up data from a USB drive and **[Previous Counts]** looks up data stored directly on the LUNA-II™.

Insert a USB drive into the LUNA-II™. Press **[Review Files]** to select a LUNA-II™-generated folder from the USB drive. The cell count results and corresponding image will appear on the right side of the screen.

Review		Protocol	DEFAULT
		Date	16 Apr., 2017 13:12
Review Files	File name	Results	
	HL-60-08062015_1342...	[Total]	
		[Live]	
		[Dead]	
		Viability	
		Average size	
		Total cell number	
		Live cell number	
		Dead cell number	
		Dilution factor	

If available in the folder, a tagged image will appear below the results. Tap on the image to make it full size. The image may be magnified with the magnifier.

Press **[Previous Counts]** to see a list of up to 1000 previous counts and their summarized results. Data can be exported to a USB drive as individual CSV files.

Review		Protocol	DEFAULT				
		Date	16 Apr., 2017 13:12				
Review Files	Name / Date	Total Cell	Live Cell	Dead Cell	Viability	Avg. Size	Protocol
	HL-60-08062015_13...	1.06E06	9.18E05	1.38E05	87.0%	13.0	DEFAULT
	08/06/2015 13:39	230	200	30			
		1.23E06	0.00E00	1.23E06	0.0%	9.4	New Protocol
	02/06/2015 17:47	267	0	267			
		2.03E06	1.75E06	2.85E05	86.0%	6.9	DEFAULT
	01/01/1970 12:07	443	381	62			
	uuu	1.38E04	0.00E00	1.38E04	0.0%	43.9	DEFAULT
	01/01/1970 09:02	3	0	3			
		7.02E05	6.43E04	6.38E05	9.2%	19.9	DEFAULT
	01/01/1970 09:01	153	14	139			
	p4-8-22052015_145...	7.99E05	0.00E00	7.99E05	0.0%	11.0	DEFAULT
	22/05/2015 14:50	174	0	174			

Chapter 6 – Maintenance and Troubleshooting

6.1 Turning On/Off

To turn the instrument on, push the power button below the touchscreen.

It is unnecessary to turn the instrument off between uses as standby mode is activated after ten minutes of inactivity. The touchscreen will blackout in standby mode. Simply press the touchscreen or push the power button to start the LUNA-II™ up again.

Turn the instrument off at the end of each day.

To turn the instrument off, press the power icon in the main menu (see Section 2.2: Startup/Main Menu) or push the power button for five seconds.

6.2 Cleaning

Turn the LUNA-II™ off and disconnect the power cable before cleaning. Make sure that liquids do not enter any part of the instrument during cleaning.

Clean the surfaces of the instrument with a soft cloth dampened with distilled water. Wipe dry immediately. Do not pour or spray liquids directly onto the instrument. Do not wet electrical wires or connections in order to avoid electrical shock or damage.

Clean the touchscreen with a soft cloth lightly dampened with an authorized LCD cleansing detergent. Wipe dry immediately. Do not exert excessive force or pressure as this can damage the resistive touchscreen.

Do not use abrasive cloths or bleach solutions as this can cause topical damage.

6.3 Installing Printer Paper

Pull the lever below the printer up to open the printer cover and reveal the paper receptacle.

Place the roll of receipt paper into the paper holder so that the end of the roll feeds from the top.

Pull the end of the printer paper roll out, and then close the printer cover.

Pull and tear the excess paper extending out of the printer.

6.4 Troubleshooting

Problem	Possible Cause	Solution
	Clumped cells	Gently but thoroughly pipette your cell suspension to break up aggregates prior to counting. Alternately, increase trypsinization time.
	Too few or too many cells	Cell concentrations of 5×10^4 - 1×10^7 cells/mL are optimal for counting. Dilute or concentrate cell suspensions accordingly.
	Improper slide insertion	Make sure that the slide has been inserted into the instrument.
Inaccurate cell count	Improper sample loading	Do not over- or under-fill the slide chambers. Carefully load the chambers with 10-12 μ L of cell suspension.
	Malfunction of optical components	Optical components may be dirty or damaged. Please contact your local distributor or Logos Biosystems.
	Damaged or contaminated slide	Use a new LUNA™ Cell Counting Slide or clean the LUNA™ Reusable Slide and its coverslips thoroughly before use. Wear gloves and handle by the edges to avoid smudging and contamination.
	Incorrect dilution factor	Adjust the dilution factor in the selected protocol or create a new protocol. Make sure the appropriate staining option has been selected.
Data transfer and saving	Incompatible USB drive	Some USB devices are undetectable or incompatible. Use the USB supplied with the instrument or use a USB 2.0.
	Too many files in the USB drive	Delete or transfer files.
Background calibration taking too long	Freezing during background calibration	If calibration takes more than 10 minutes, reset the system by turning the power off and then on again. Contact your local distributor or Logos Biosystems if calibration fails repeatedly.
	Incompatible USB drive	Some USB devices are undetectable or incompatible. Use the USB supplied with the instrument or use a USB 2.0.
Errors while updating software	More than one software version on the USB drive	Delete previous versions of software from the USB drive before downloading new software.
	Incorrectly saved or damaged software	Use the supplied or a compatible USB drive. Download the file again into the root directory of the USB drive. Insert the USB and press [Software Updates] in the Settings screen. If the problem persists, contact your local distributor or Logos Biosystems.
LED on printer flashing	Paper receptacle empty	Check to see there is enough paper in the printer. If there is not, replace it (see Section 6.3: Installing Printer Paper). If the flashing persists, contact your local distributor or Logos Biosystems.

Chapter 7 - Ordering Information

Cat #	Product	Quantity
L40001	LUNA-II™ Automated Cell Counter (with printer)	1 unit
L40002	LUNA-II™ Automated Cell Counter (without printer)	1 unit
L12008	LUNA™ Reusable Slide	1 unit
L12010	LUNA™ Reusable Slide Coverslips	10 units
L12001	LUNA™ Cell Counting Slides, 50 slides (100 counts)	1 box
L12002	LUNA™ Cell Counting Slides, 500 slides (1,000 counts)	10 boxes
L12003	LUNA™ Cell Counting Slides, 1,000 slides (2,000 counts)	20 boxes
T13001	Trypan Blue Stain, 0.4%	2 x 1 mL
L13002	Erythrosin B Stain	2 x 1 mL
B13001	LUNA™ Standard Beads	2 x 1 mL
P12002	LUNA-II™ Printer Paper (10/pk) – minimum 700 prints	1 pack
U10005	USB Drive, 16 GB	1 unit

Chapter 8 - Purchaser Notification

8.1 Limited Use Label License: Research Use Only

The purchaser of this product should use this product only for research for the sole benefit of the purchaser. By use of this product, the purchaser agrees to be bound by the terms of this limited use statement whether the purchaser is a for-profit or a not-for-profit entity.

If the purchaser is not willing to accept the conditions of this limited use statement and this product is unused, the Company will accept return of the product with a full refund.

The purchaser cannot resell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party for Commercial Purposes.

Commercial Purposes mean any and all uses of this product and its components by a party for monetary or other consideration, including but not limited to, (a) product manufacture, (b) providing a service, information, or data, (c) therapeutic, diagnostic, or prophylactic purposes, or (d) resale of this product or its components whether or not such product and its components are resold for use in research.

Logos Biosystems, Inc. ("Company") will not claim any consideration against the purchaser of infringement of patents owned or controlled by the Company which cover the product based on the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine, or prophylactic product developed in research by the purchaser in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product.

For any use other than this limited use label license of research use only, please contact the Company or email info@logosbio.com for more information.

8.2 Instrument Warranty

Logos Biosystems, Inc. ("Company") warrants to the original purchaser ("Purchaser") that the instrument ("Instrument"), if properly used and installed, will be free from defects in materials and workmanship and will conform to the product specifications for a period of one (1) year ("Warranty Period") from the date of purchase. If the Instrument under this limited warranty fails during the Warranty Period, the Company, at its sole responsibility, will:

- 1) within and up to 30 calendar days of purchase, refund the purchase price of the Instrument to the Purchaser if the Instrument is in original conditions; or,
- 2) after 30 calendar days of purchase, only replace or repair the Instrument for up to the Warranty Period without issuing a credit.

In no event shall the Company accept any returned instrument (including its components) that might have been used or contaminated in some labs, including but not limited to, HIV or other infectious disease or blood-handling labs. This limited warranty does not cover refund, replacement, and repair incurred by accident, abuse, misuse, neglect, unauthorized repair, or modification of the Instrument. This limited warranty will be invalid if the Instrument is disassembled or repaired by the Purchaser.

In case that the Company decides to repair the Instrument, not to replace, this limited warranty includes replacement parts and labor for the Instrument. This limited warranty does not include shipment of the Instrument to and from service location or travel cost of service engineer, the costs of which shall be borne by the Purchaser. Every effort has been made to ensure that all the information contained in this document is correct at its publication. However, the Company makes no warranty of any kind regarding the contents of any publications or documentation as unintended or unexpected errors including occasional typographies or other kinds are inevitable. In addition, the Company reserves the right to make any changes necessary without notice as part of ongoing product development. If you discover an error in any of our publications, please report it to your local supplier or the Company. The Company shall have no responsibility or liability for any special, incidental, indirect or consequential loss or damage resulting from the use or malfunction of the Instrument.

This limited warranty is sole and exclusive. The Company makes no other representations or warranties of any kind, either express or implied, including for merchantability or fitness for a particular purpose with regards to this Instrument. To obtain service during the Warranty Period, contact your local supplier or the Company's Technical Support team.

OUT OF WARRANTY SERVICE

Please contact your local supplier or the Company's technical support team in order to obtain out-of-warranty service. If necessary, repair service will be charged for replacement parts and labor hours incurred to repair the Instrument. In addition, the Purchaser is responsible for the cost of shipping the Instrument to and from the service facility and, if necessary, the travel cost of a service engineer.



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