logos biosystems

LUNA III™

Automated Cell Counter

User Manual

DISCLAIMER

The contents of this document are subject to change without notice.

The LUNA-III™ 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

The trademarks used in this document are the property of Aligned Genetics, Inc.

Logos Biosystems is the brand name of Life Science business of Aligned Genetics, Inc.

CERTIFICATION MARKS

	The WEEE (Waste Electrical and Electronic Equipment) symbol indicates that users of this instrument have 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)
	This device complies with Part 15 of the FCC Rules.
	The KC certification mark indicates that this instrument conforms with Korea’s product safety requirements for electrical and electronic equipment and components for which this marking is required.

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Chapter 1 – Introduction

1.1 Product Overview

The LUNA-III™ Automated Cell Counter is an image-based cell counting device that features an innovative autofocusing 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-III™ takes care of the rest, eliminating the subjectivity and time expenditure of manual cell counting.

The LUNA-III™ counting algorithm accurately 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-III™ provides:

- the concentration of total, live, and dead cells per mL,
- the number of total, live, and dead cells ,
- 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-III™ 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-III™ also provides the option to review previous data.

The LUNA-III™ is compatible with both disposable and reusable slides. The LUNA™ Reusable Slide works seamlessly with the LUNA-III™ as well as other LUNA™ family cell counters, including LUNA-FX7™, LUNA-FL™, and LUNA-STEM™, for both brightfield and fluorescence cell counting. It is designed to offer cost-efficient and precise cell counting, combining the affordability of manual cell counting with the speed, accuracy, and convenience of automated cell counting. On the other hand, the disposable LUNA™ Cell Counting Slides provide an optimal counting experience for those looking for the highest level of accuracy with easy maintenance.

1.2 Key Features

Key Features	Description
Compact, space-efficient design	Lightweight and compact, the LUNA-III™ 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 & autoexposure	High-precision stepper motor 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, counting cells takes 10 seconds. With autofocusing, it takes just 15 seconds.
Reanalysis	Raw images may be reanalyzed using a different protocol.
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.
Internal memory	10GB. Up to 400 counts can be saved directly when the file is 25MB.
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-III™ product package contains the following components.

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

* Optional LUNA™ Printer II (P17001) can be used with *LUNA-III™ Automated Cell Counter.*

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-III™ 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	1.8 kg (4.0 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

LUNA-III™ Automated Cell Counter

The front of the LUNA-III™ 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.

Front view

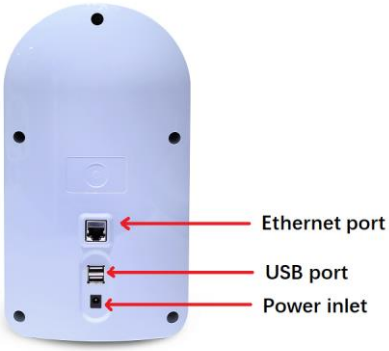
Right side

USB ports

USB ports allow the user to transfer or print cell count data. Data may be transferred via USB drive. Counting data may be printed using the LUNA™ Printer II (P17001).

Ethernet port

The Ethernet port allows the instrument to be connected to a computer network and user can transfer counting data through the network.



Back

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

LUNA-III™ Cell Counting Slides

The LUNA-III™ is compatible with the Reusable Slide formats in addition to the standard LUNA™ Cell Counting Slides and Validation slide.

Channel No.	2 Channel	Reusable
Compatible Slides	LUNA™ Cell Counting Slides	LUNA™ Reusable slides
		
Sample Throughput	Up to 2 samples	1 sample
Sample Loading Volume	10 µL/chamber	10 µL/chamber
Analysis Volume	0.5 µL/chamber	0.5 µL/chamber

LUNA-III™ Cell Counting Reagents

The LUNA-III™ is compatible with Trypan Blue and Erythrosin B staining reagents listed below for brightfield cell counting.

Cat #	T13001	T13011	L13002
Compatible Reagents	Trypan Blue Stain, 0.4%	Trypan Blue Stain, 0.4%, Sterile-filtered	Erythrosin B Stain
			
Qty	1mL×2	1mL×2	1mL×2
Storage	Room temperature	Room temperature	Room temperature

1.6 General Guidelines

Follow the instructions below to obtain the best results with the LUNA-III™ 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 than Trypan Blue.

3. As the LUNA-III™ is calibrated before shipping, recalibration before use is not necessary. See Section 2.3: Settings: Background Calibration to see when background calibration is necessary.

Chapter 2 – Setting up

2.1 Installation

Installation

Place the LUNA-III™ on a clean, level and sturdy surface.

- Avoid vibrations from other devices.
- Do not install the instrument in a location that will expose the device to intense ultraviolet light.
- Allow at least 5 cm (2 inches) free space at the back of the instrument to prevent overheating of the instrument.

Connect the instrument to electrical outlets using the supplied power cord and AC adapter.

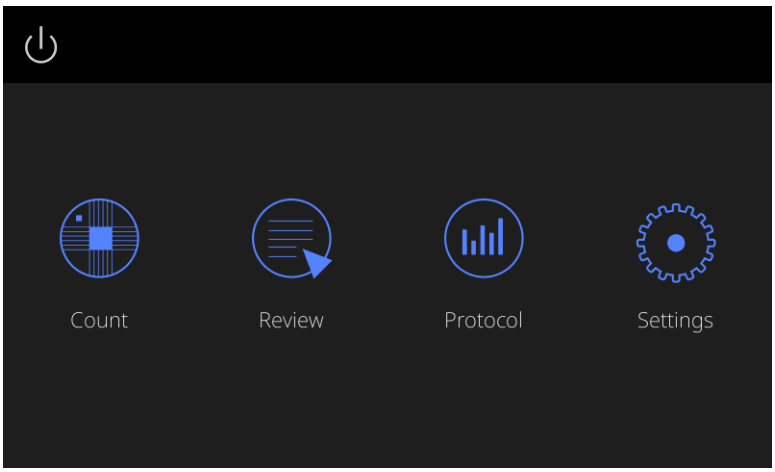
- Make sure the power cords are appropriate for your region.
- Always use power cord and AC adapter provided or approved by Logos Biosystems. If appropriate cord is not used, the electrical safety of the instrument cannot be guaranteed.

2.2 Startup/Main Menu

LUNA-III™ Startup

Push the power button located below the touchscreen to turn on the instrument. After a short beep, the company logo will appear, followed by the home screen.

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



For instructions on when and how to turn the instrument on or off, see Section 7.1: T urning On/Off.

Screen Saver

The screen backlight will automatically turn off after 10 minutes of inactivity. Touching the screen will reactivate the instrument.

2.3 Settings

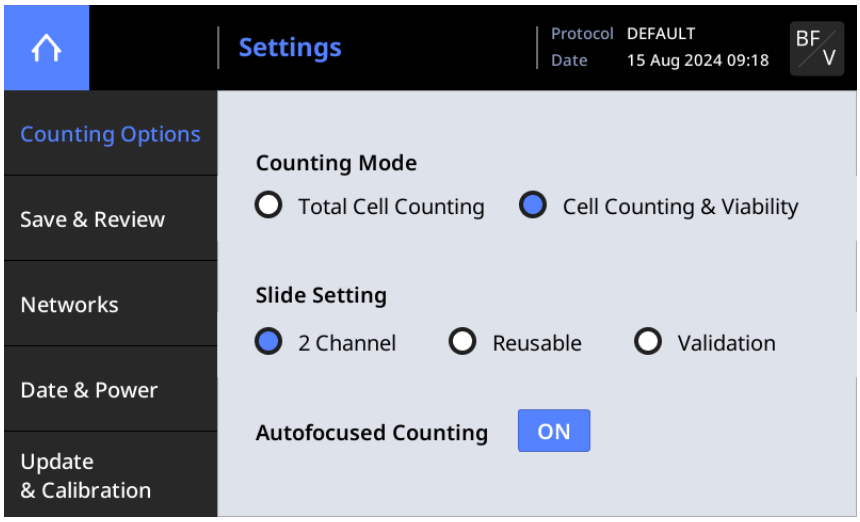
Settings

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.

Within Settings, software updates, date and time changes, and background calibrations may be performed. Additionally, options for ‘Counting Options’, ‘Save & Review’ and ‘Network’ may be adjusted.

Counting Options



Counting mode

Select Total Cell Counting mode or Cell Counting & Viability mode.

- Total Cell Counting mode is used to enumerate total cell numbers without staining cells.

- Cell Counting & Viability mode is used to count cells and calculate the viability of cells stained with Trypan Blue Stain, 0.4% (T13001) or Trypan Blue Stain, 0.4%, Sterile-filtered (T13011) or Erythrosin B Stain (L13002).

Slide setting

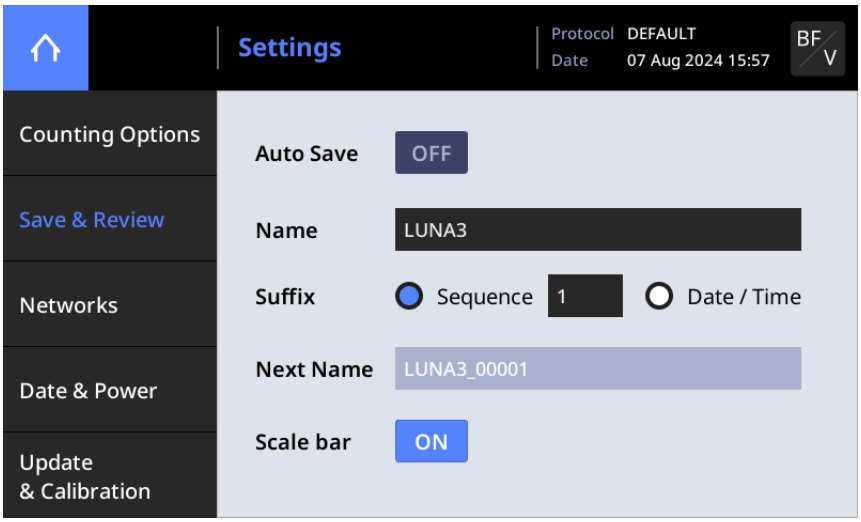
Choose appropriate slide format.

Autofocused counting

When Autofocused counting is activated, LUNA-III™ will readjust the focus during image capture (recommended to keep active).

Save & Review

Press **Save & review** on the left menu.



Auto Save

When Auto Save is activated, cell counting results are automatically saved according to the Name and Suffix rule.

Auto Save rule

- **Name**

  This name will serve as the prefix for all saved counts.

- **Suffix**

  Select **Sequence** to automatically add sequential numbers to the prefix name; OR, select **Date/Time** to automatically append date and time to the prefix name.

- **Next name**

  Displays file name to be used for the next count to be saved.

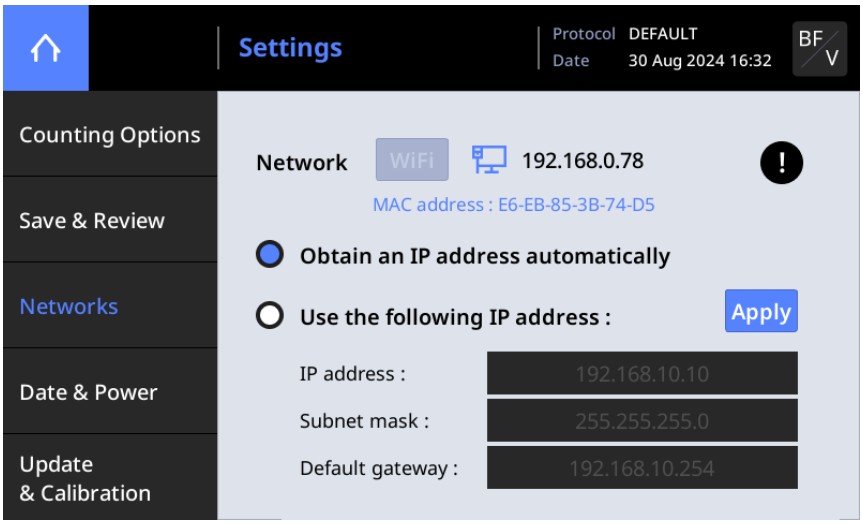
Scale bar

Includes or excludes scale bar for Tag (Analyzed) images.

Networks

The LUNA-III™ may be connected to a local network via Ethernet.

Within **SETTINGS**, press **NETWORK**.



Ethernet connection

Connect an Ethernet cable to the instrument.

When connected, an IP address will appear on the screen.

MAC Address

Available as a network address when you access the LUNA-III™.

- When assigning a static IP

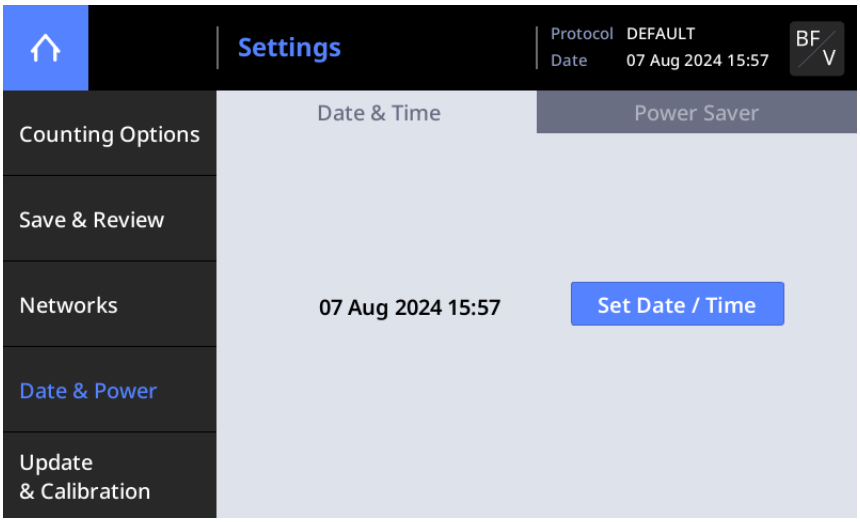
Date & Power

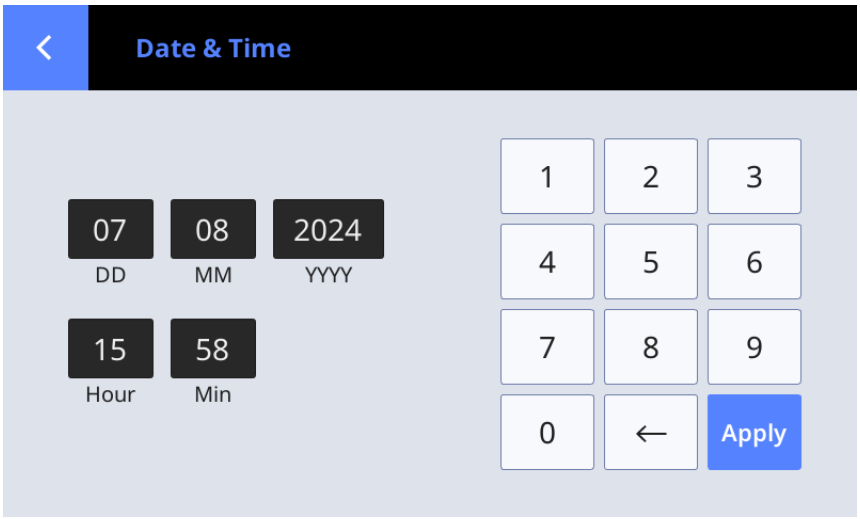
Date & Time

The LUNA-III™ uses a 24-hour clock that is preset to Korean Standard Time. Adjust the settings to the local date and time.

Press **DATE & POWER**.

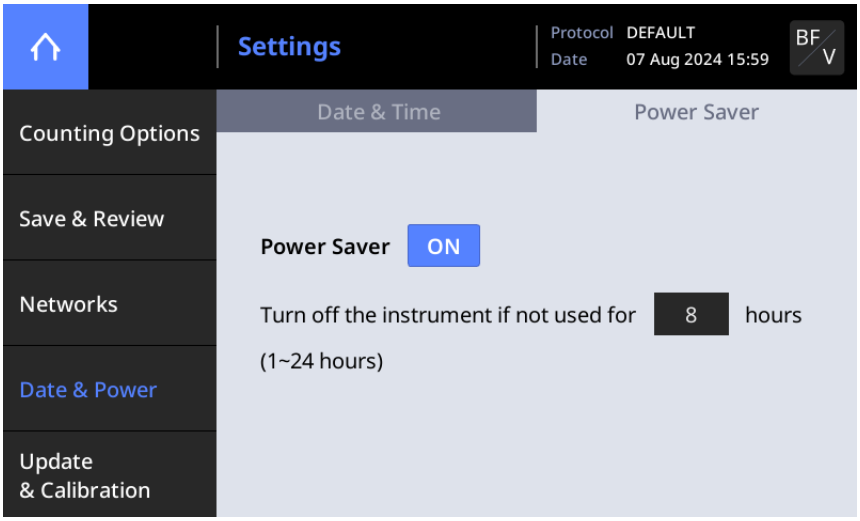
Press **Set Date & Time**. Input the desired values. Press **APPLY** to save changes.





Power saver

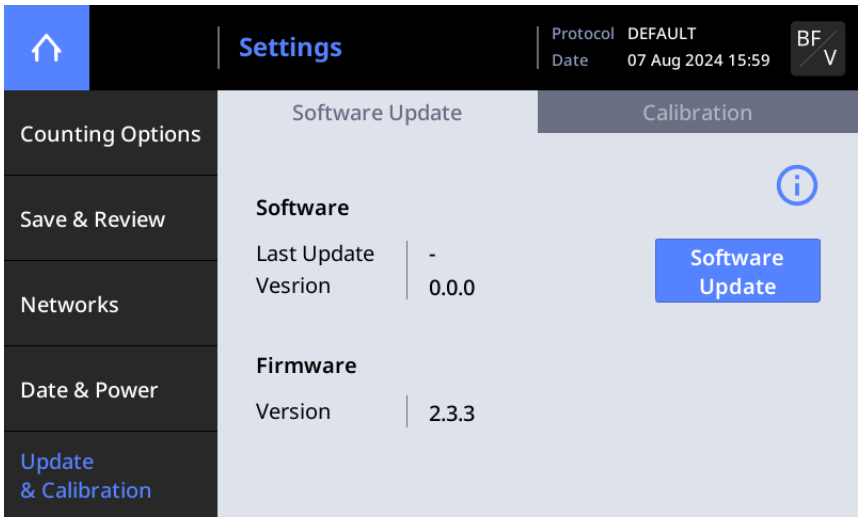
LUNA-III™ provides a **Power saver** to save energy and protect environment. Activate Power saver to automatically shut down the LUNA-III™.



Update & Calibration

Software Update

Logos Biosystems continually provides software updates to ensure optimal performance. The existing version of software is displayed in **Settings: Update & Calibration**.



1. The most recent version may be downloaded from the Logos Biosystems website (www.logosbio.com) into the root directory of a compatible USB drive.

2. Press [Software Update] in the Settings screen.

3. Insert the USB drive with the downloaded file and authentication key into a USB port.

4. Press Software update.

5. Press Start. If a software update has been found, press OK.

6. Press Restart, then the instrument will automatically shut down and then restart.

7. Prior to the next count, perform calibration.

! **Important!** Users must recalibrate the background after each software update (see Section 2.3: Settings: Background Calibration)

Background Calibration

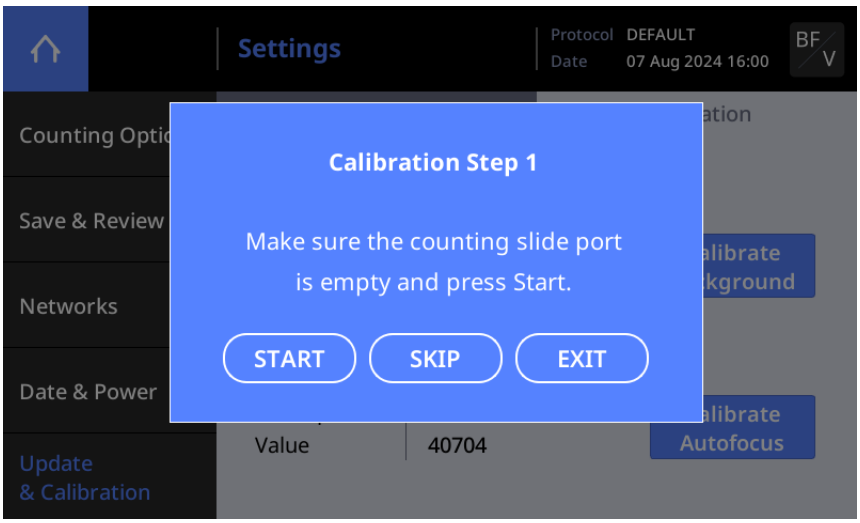
Background calibration adjusts for the specific brightness of the stain used for counting and is a prerequisite for successfully detecting cells. Users must recalibrate the background after each software update. The LUNA-III™ is optimized for use with trypan blue or Erythrosin B.

To perform calibration:

1. Press **Calibrate Background**.

! **Important!** Do not turn off the instrument during the calibration process.

2. A window will appear with directions for **Calibration Step 1**.

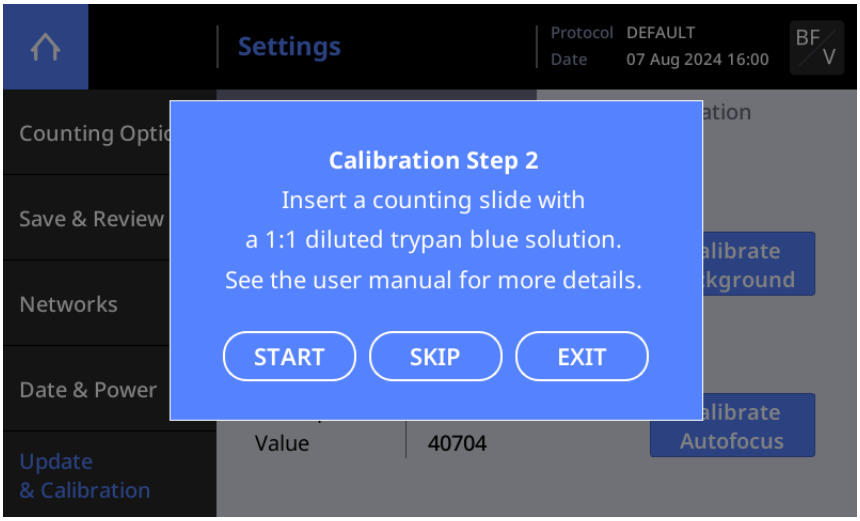


3. Remove any counting slide from slide port.

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.

4. Press **START** and wait for a couple of seconds.

6. The Calibration Step 2 window will appear upon completion.



7. Mix one part staining dye with an equal volume of plain media, water, or PBS without cells or particles. Load 10 µL of the diluted stain into the chamber of a new LUNA™ Cell Counting Slide or a clean LUNA™ Reusable Slide.

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

! **Important!** Do not insert the slide facedown.

9. Press **START.** Do not remove the slide or turn off the instrument during this process or, press **SKIP**, if this calibration is not needed.

11. Upon completion, the background calibration value will be updated in the Settings Screen.

Autofocus Calibration

Autofocus calibration aligns the center position of the slides to obtain precise autofocused imaging. Users must perform autofocus calibration when the autofocused image appears blurry or after the instrument had optic-related repairs.

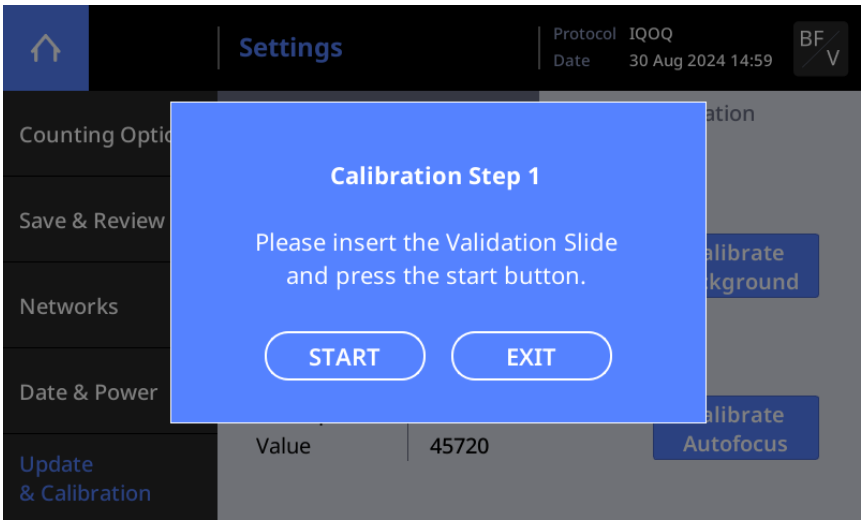
To perform calibration:

1. Before starting the autofocus calibration, you need to prepare the Validation Slide.

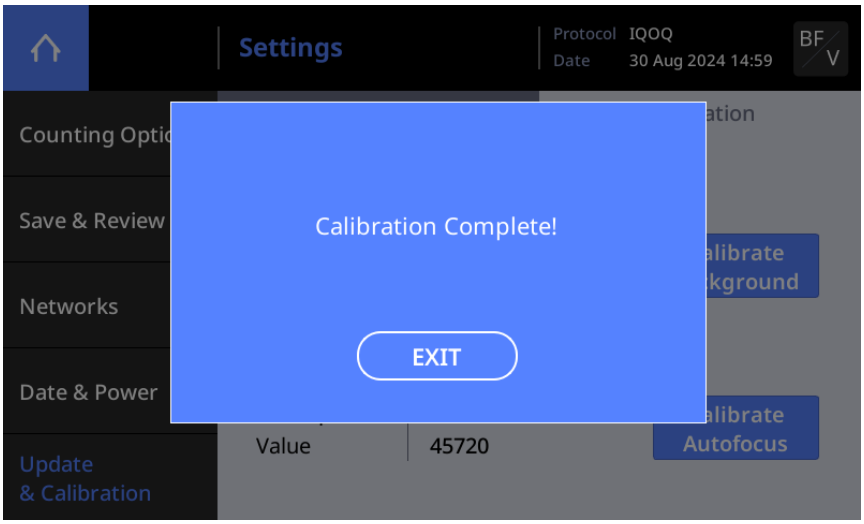
* Validation Slide: Cell Counter Validation Slide-BF II(Cat# L72041)

2. Insert the slide face up into the counting slide port.

3. Press **START** and wait for a couple of seconds. Do not remove the slide or turn off the instrument during this process.



4. Upon completion, the calibrated focus value will be updated in the Settings Screen.



Prepare a counting slide and load the LUNA™ Standard Beads or your own cell sample.
Go back to the count screen and insert the prepared slide.
Use the Autofocused Counting to check if the autofocus works well.

Chapter 3 – Setting the Count Protocol

3.1 Protocol Selection

Default Protocol

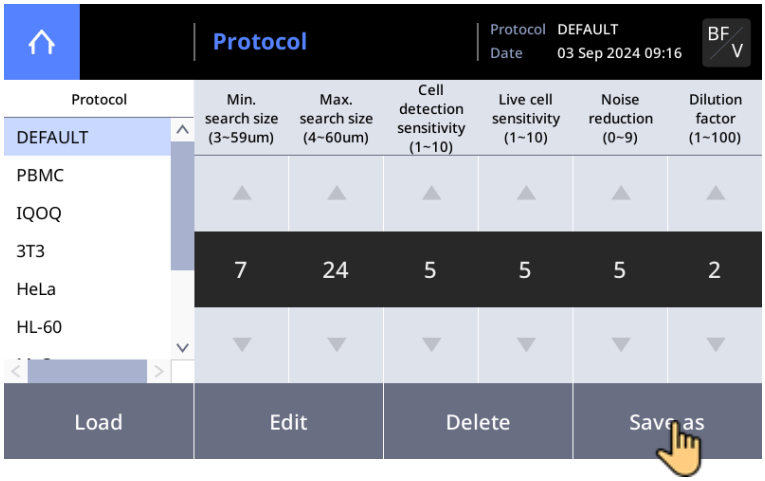
Each counting mode comes with the following pre-set counting protocols. These protocols cannot be edited.

Counting mode	
Bright Field Cell Counting -Total Cell Counting	**Bright Field Cell Counting -Cell Counting & Viability**
DEFAULT	DEFAULT
PBMC	PBMC
	IQOQ
	3T3
	HeLa
	HL-60
	McCoy
	RAW264_7
	U-937

The **DEFAULT** protocols, by design, will provide optimal results for most cell types, but protocols for specific cell types or applications may need to be optimized.
All pre-set counting protocols have been carefully optimized through repeated experiments, and we recommend using them initially for counting the respective cells. However, as cell conditions can vary between laboratories, further optimization may be necessary.

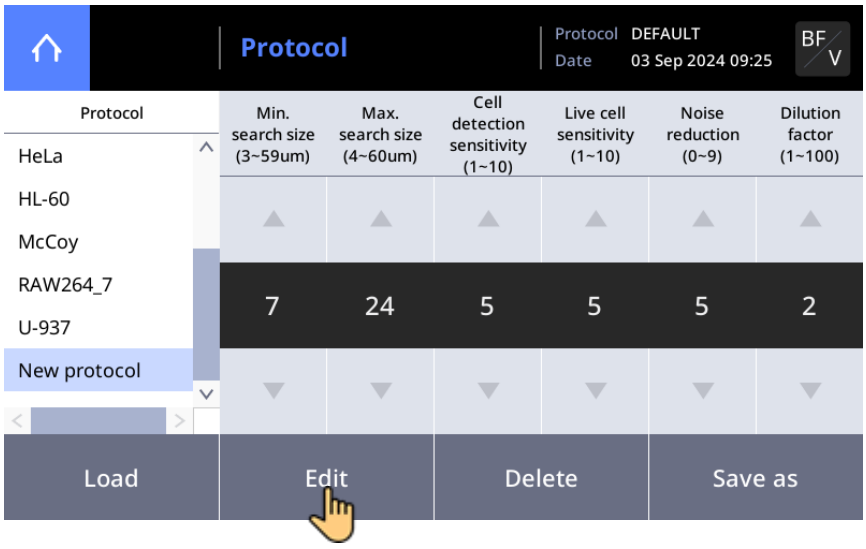
Create Protocols

Customized protocols for specific cell types may be created. To create a new protocol, select any protocol and press **Save as**.



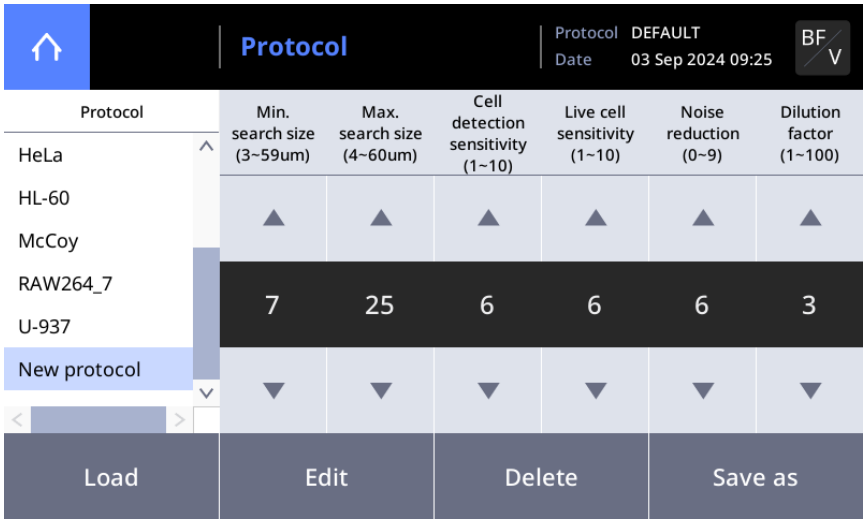
Rename the protocol and press **Save**. The newly created protocol will appear in the list of protocols.

Edit Protocols



Select a protocol that is not the *Default* protocol.

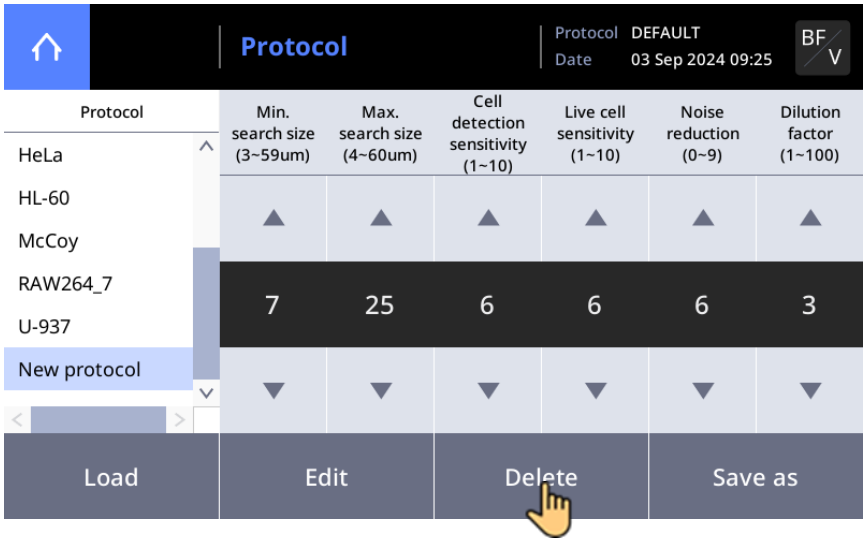
Press **Edit**. This will activate the arrows for each parameter, turning them black.

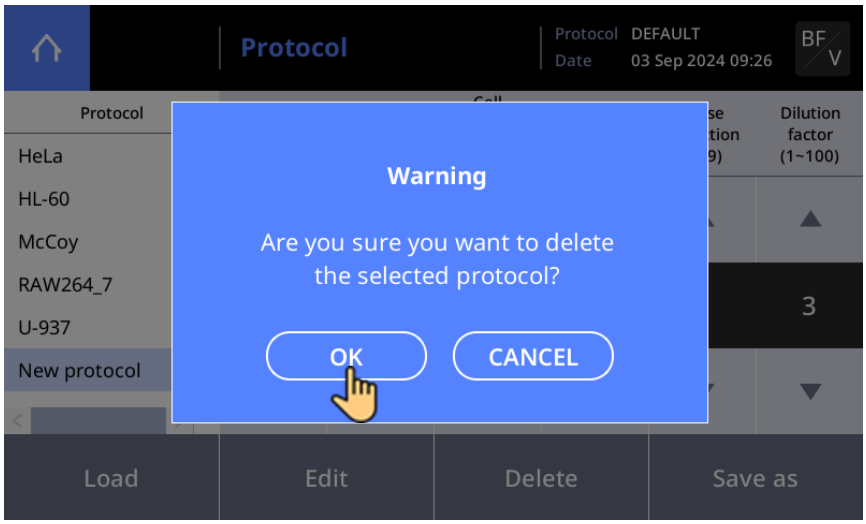


Press the arrows to adjust the values of each parameter. Press **Save as** to change the protocol name. Press **Load.** Save the edited protocol under the selected name and activate it.

Delete Protocol

Select the protocol to delete and press **Delete**.

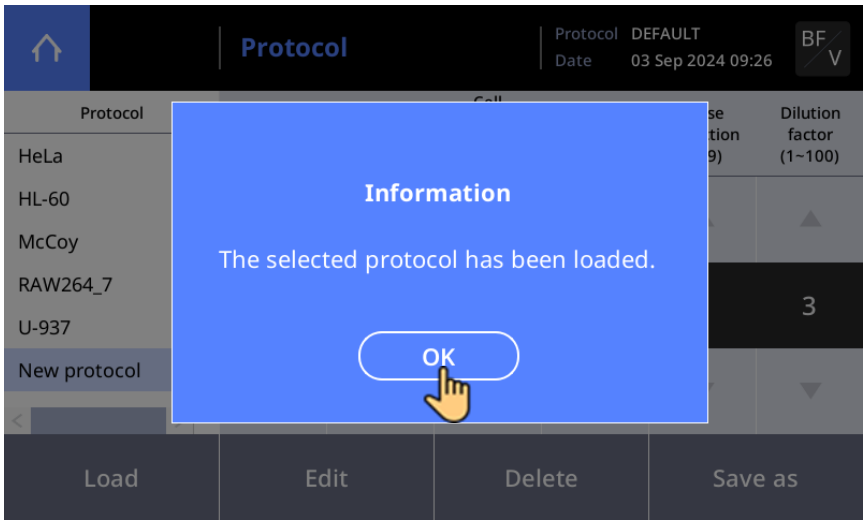




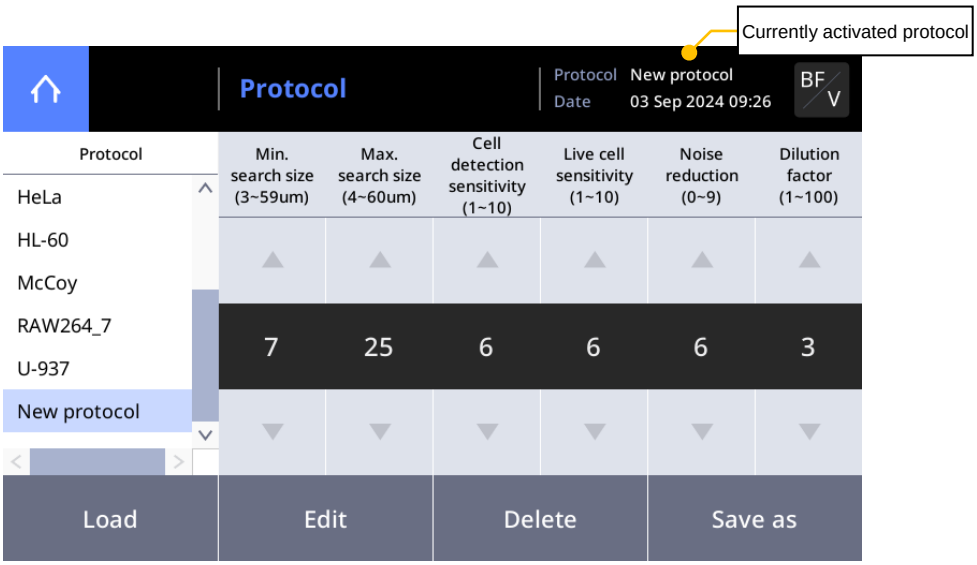
Press **OK**. The selected protocol is deleted.

Load Protocol

Select the desired protocol and press Load.

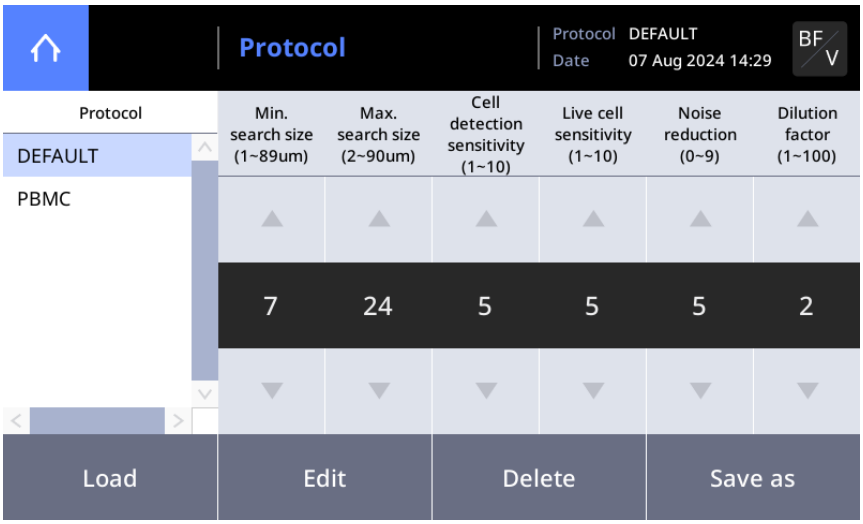


The current active protocol name can be seen in the upper right-hand corner of the screen.



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**.



3.2 Protocol Parameters

Brightfield Cell Counting Parameters

Parameters for [Brightfield cell counting-Total cell counting]

	Min. search size (μm)	Max. search size (μm)	Cell detection sensitivity	Noise reduction	Dilution factor
Range	3-59	4-60	1-10	0-9	1-100
DEFAULT	7	24	5	5	1
PBMC	7	15	5	4	1

Parameters for [Brightfield cell counting-Cell counting & Viability]

	Min. search size (μm)	Max. search size (μm)	Cell detection sensitivity	Live cell sensitivity	Noise reduction	Dilution factor
Range	3-59	4-60	1-10	1-10	0-9	1-100
DEFAULT	7	24	5	5	5	2
PBMC	7	15	5	6	4	2
IQOQ	7	24	5	5	5	1
3T3	10	35	5	5	6	2
HeLa	12	27	5	5	5	2
HL60	8	21	5	5	5	2
McCoy	10	25	5	5	5	2
RAW264_7	8	20	5	5	5	2
U937	8	20	5	5	5	2

Min./Max. search size

"Search size" refers to the approximate cell size that the algorithm uses to identify potential cell objects. Therefore, the more accurately you input the minimum and maximum search size for the cells, the more precise counting results are expected to be.

Even for cells that are relatively uniform in size, variations can occur due to the cell cycle or cell status. Hence, it is recommended to set the minimum search size 5 to 10 µm smaller than the expected average cell size, and the maximum search size 5 to 10 µm larger. For example, if the average cell size is 15 µm, it is recommended to set the search size range between 10 to 20 µm or 5 to 25 µm.

Setting a narrower search size allows for the identification of cells that more closely match the expected size, but increases the possibility of missing cells outside the search range. Conversely, setting a broader search size enables the detection of a wider range of cell sizes, but may make it harder to accurately identify cells within clusters.

The search size set in the default protocol is chosen to effectively detect most mammalian cells. If you are not familiar with the concept of Search size, start by using the default protocol to ensure that your cells are properly detected. Then, gradually adjust the values to find the optimal search size for your specific cell types.

Cell detection sensitivity

Cell detection sensitivity refers to the sensitivity of object separation from the background. A higher Cell detection sensitivity value will increase detection of signals from weakly stained cells or smaller objects, but can also increase false positive calls.

Cell detection sensitivity is also used to distinguish individual cells within clustered cells. Increasing the cell detection sensitivity value enhances declustering, allowing a single cluster to be separated and detected as smaller, individual cells.

The cell detection sensitivity set in the default protocol is selected to effectively detect most mammalian cells. Begin by using the default protocol to ensure that your cells are properly detected, and then gradually adjust the sensitivity to find the optimal value for your sample.

Live cell sensitivity

Live cells with intact cell membranes exclude Trypan blue, Erythrosin B. The dyes stain the cytoplasm of dead cells with compromised membranes. As a result, object intensity of unstained live cells is brighter than the stained dead cells. A higher Live cell sensitivity will decrease the intensity cutoff value and increase the number of live cells detected.

Live Cell Sensitivity is not available in the protocol of the Total cell counting mode.

Noise reduction

This option allows for the adjustment of background noise during counting. With more noise reduction, the instrument will not detect weakly stained objects. With lower noise reduction, the instrument can detect objects with fainter signals.

Dilution factor

The dilution factor is used to calculate cell concentrations accurately. The default dilution factor is preset as 1 for Total cell counting and as 2 for Total cell & viability counting (assuming a 1:1 ratio of stain to cell suspension).

This value can be modified according to the dilution of the original sample in increments of 1 between 1-10 and, increments of 10 between 10 -100. For users handling highly dense cell cultures. For highly dense cultures, serial dilutions and several counts with appropriately adjusted dilution factors may be necessary.

Chapter 4 – Counting Cells

4.1 Sample Preparation

Sample Staining

Brightfield cell counting

For cell counting and viability, prepare a cell suspension according to standard procedures. Mix gently but thoroughly to ensure that the suspension is homogenous. Mix the sample, 1:1, with Trypan Blue Stain, 0.4% (T13001) or Trypan Blue Stain, 0.4%, Sterile-filtered (T13011) or Erythrosin B Stain (L13002). Mix gently, but thoroughly to ensure a homogenous suspension.

For total cell counting, load the sample directly onto the slide without staining the sample.

Prepare a new LUNA™ Cell Counting Slide or a clean LUNA™ Reusable Slide. Hold the slide by its edges and load 10 ~ 12 µL 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.

Sample Loading

Load the appropriate volume for each slide chamber according to the table below:

LUNA™ Cell Counting Slides	LUNA™ Reusable Slides
 10 ~ 12 µL	 10 ~ 12 µL

4.2 Counting with the LUNA-III™

Slide Insertion

Insert the slide face up and sample-side first into the counting slide port of LUNA-III™. The LUNA-III™ 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.

Viewing Images

Focus

Use the up & down arrows on the focus control bar to manually adjust the focus.

Find Cells

Press the **Find Cells** to simultaneously perform Autofocus and Autoexposure.

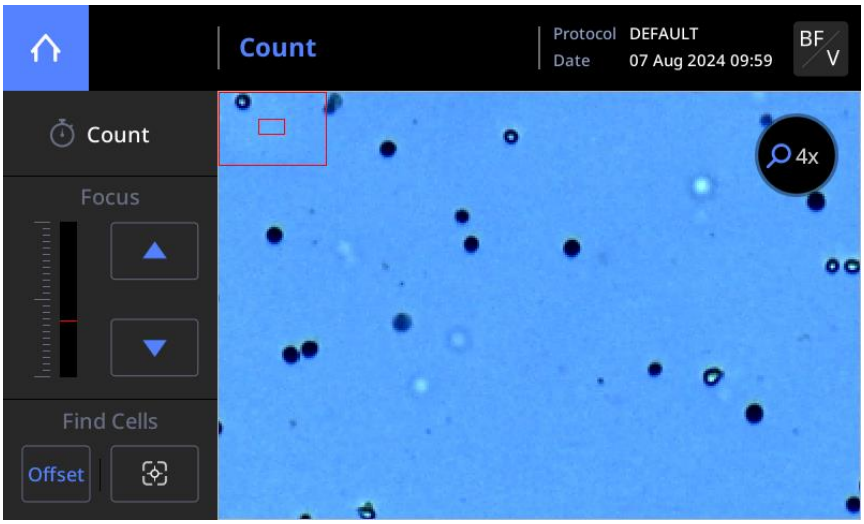
Offset

Due to the characteristics of the cell, the autofocus result may be constantly shifted from the focal plane. The offset function allows accurate focus to be obtained by shifting the focal plane by the offset value.

Zoom

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.

Cell Counting



Press [Count] to start counting.

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

Counting time will vary depending on slide type, counting mode and the protocol used. With the DEFAULT protocol, cell samples with a concentration of ~1 x 10^6 cell/mL will take at minimum 10 seconds to count without autofocusing or 15 seconds with autofocusing.

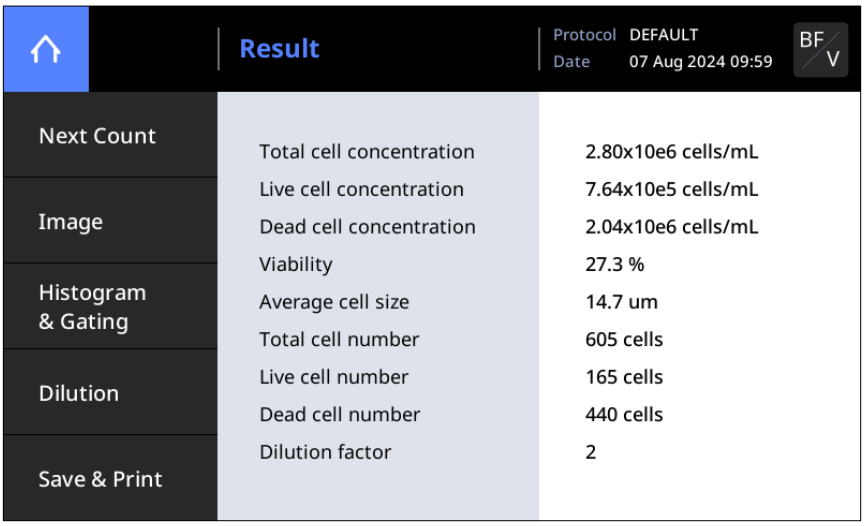
Total Cell Counting results will appear.

4.3 Results

Results

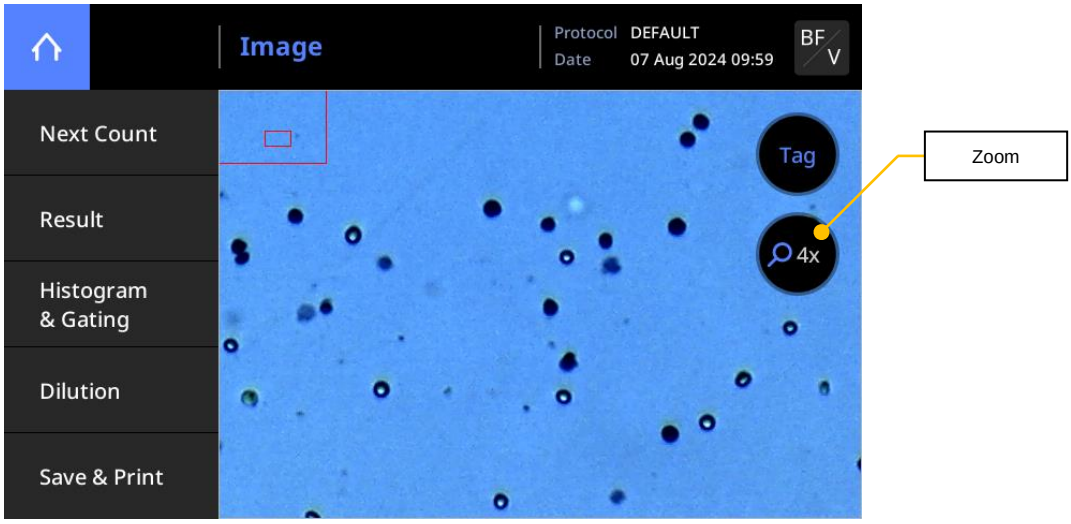
The LUNA-III™ has onboard data analysis software that allows users to analyze cell count and viability data immediately.

After counting is complete, the data will appear in the **RESULT** window.



Image

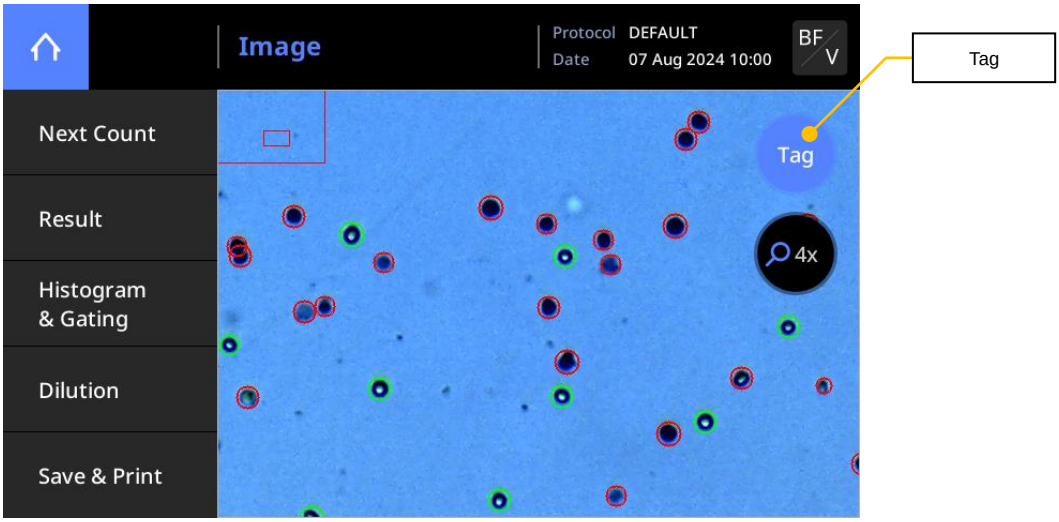
Press [**Image**] to view the captured image of the analyzed cell sample.
Press [**Result**] to return to the result screen.



Use a finger or a stylus to navigate the image. The **Tag** and magnifier buttons are to the right of the image.

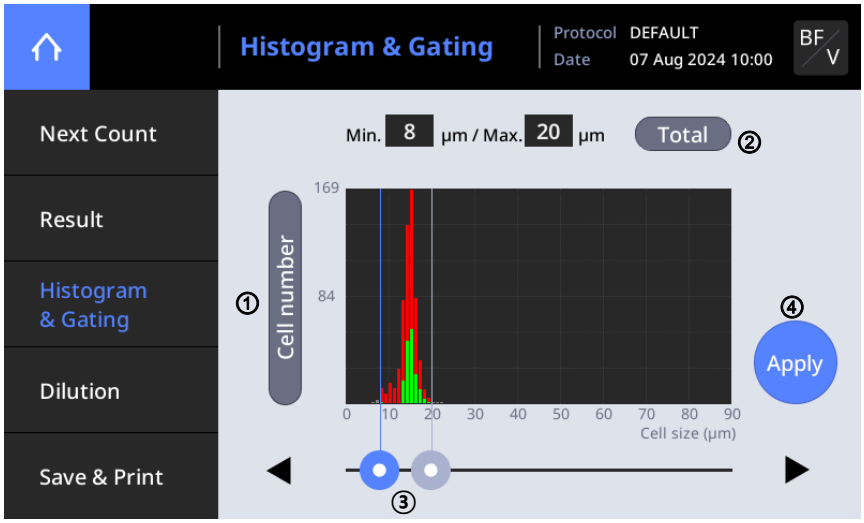
Press the magnifier button to zoom in and out of the saved image.

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.



Histograms

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



① Cell concentration or number can be graphed according to cell size.
To toggle between cell concentration, cell cluster, and cell number press the Y-Axis title.

② Each histogram for total, live, and dead cells can be displayed.
To switch between total, live, and dead, press the title box.

③ Cell size gating parameters may be changed by pressing the slider circle icon. An active slider will be highlighted in blue. Press the arrows on either end of the size to alter the minimum and maximum size limits.

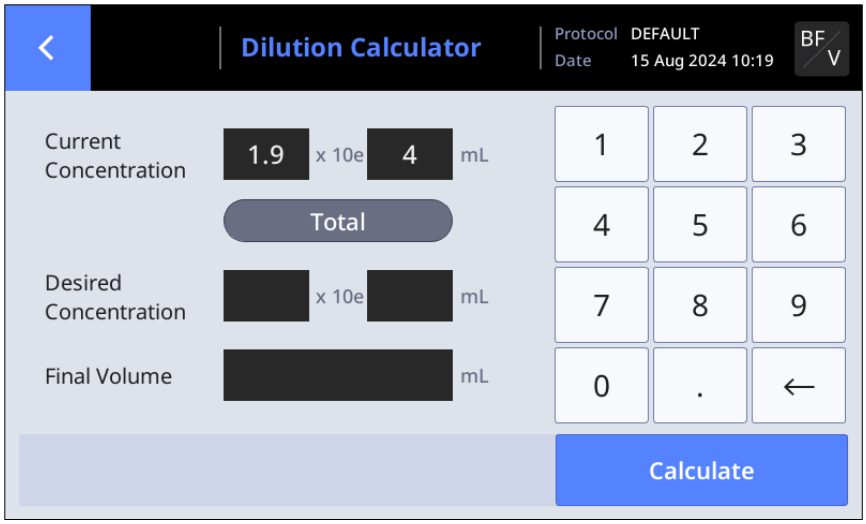
④ Press **Apply** to set cell size gating parameters. Counting results will adjust accordingly.

The gating function is helpful for monitoring co-cultured cells with distinct sizes and the exclusion of noncellular particles.

Dilution Calculator

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

Press **[Dilution]** to open the Dilution Calculator.

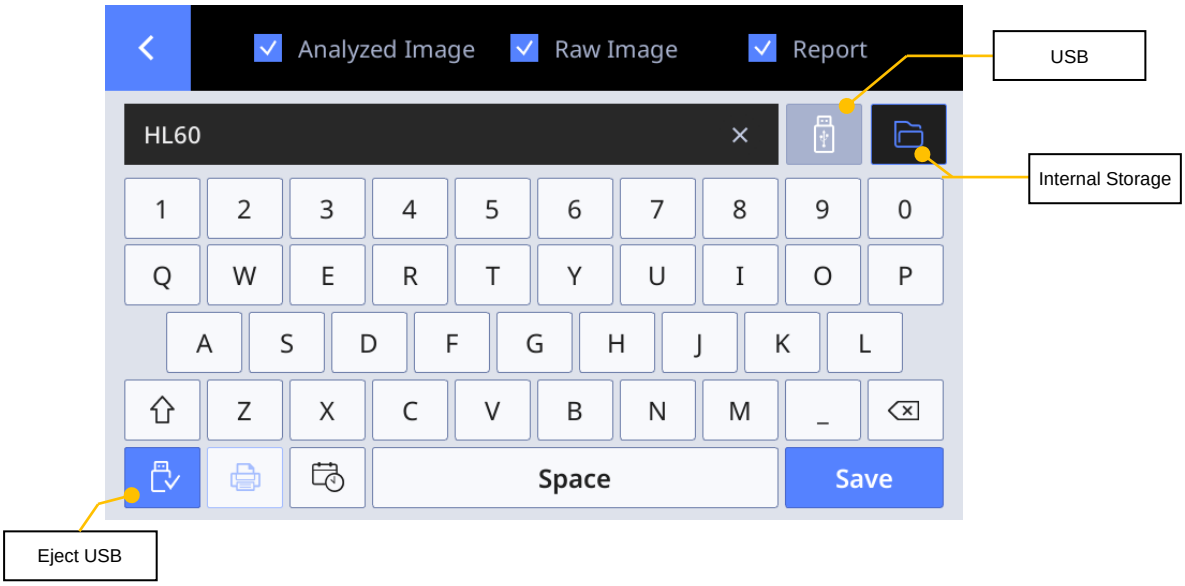


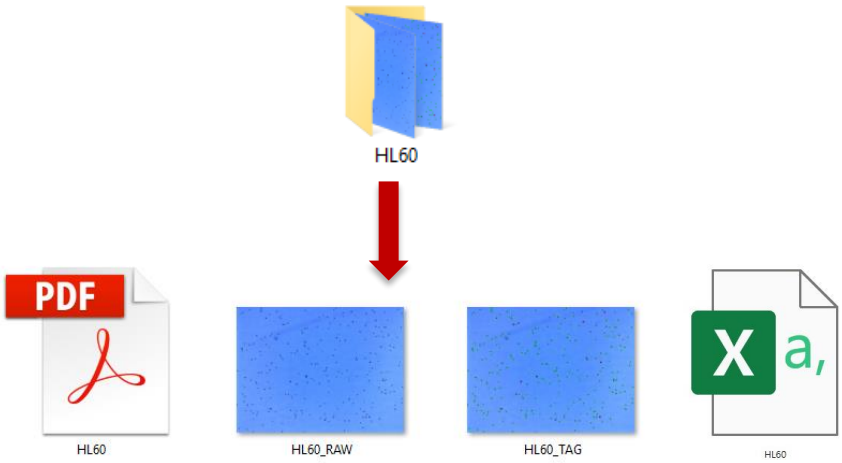
The dilution calculator starts out with the concentration of total cells (live and dead) as the current concentration. The current concentration options are **Total, Live, Dead**, and **Custom**, allowing users to set the current concentration to be the total cell concentration, live cell concentration,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

Enter the values for the desired concentration and final volume. Press **Calculate** and dilution instruction will appear in the grey message box.

Save

Press [**SAVE & PRINT**] to open the save window.





Select the desired saving options:

Save Options	File Type	Description
Analyzed image	TIF	Tagged images of cells
Raw image	TIF	Untagged images of cells
Report	PDF	Report with data, images, and histograms

Select the icon where files are to be stored :

Save path	Description
	USB
	Internal Storage

Using the onscreen keyboard, provide the name and append the date and time by pressing the Calendar button.

Press **Save**. A folder name will be created with the name provided.

When saving, one of the following must be selected: Analyzed image, Raw image, or Report.

Eject USB Press this button before removing the USB memory to prevent data loss.

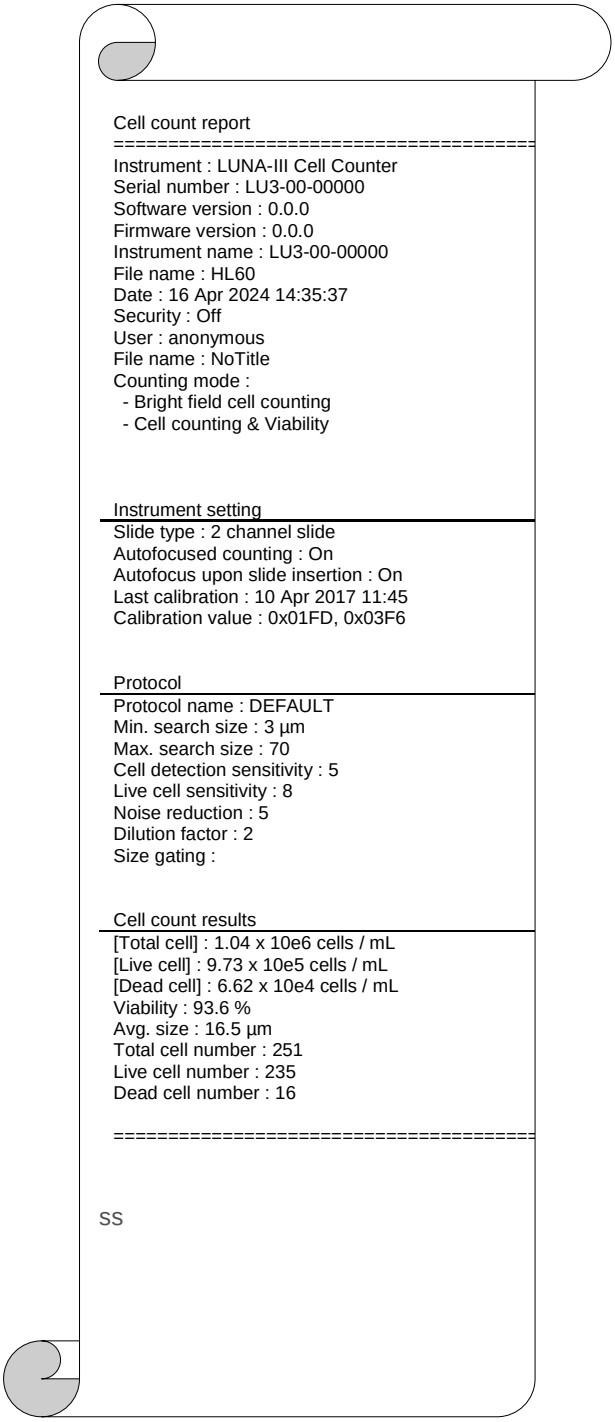
Print

To print a text summary of the counting results, make sure the thermal printer LUNA ™ Printer II (P17001) is connected to the LUNA-III™.

Press **Print**  icon.

The printer should be connected before powering on the instrument.

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



Cell count report
==========
Instrument : LUNA-III Cell Counter
Serial number : LU3-00-00000
Software version : 0.0.0
Firmware version : 0.0.0
Instrument name : LU3-00-00000
File name : HL60
Date : 16 Apr 2024 14:35:37
Security : Off
User : anonymous
File name : NoTitle
Counting mode :
- Bright field cell counting
- Cell counting & Viability

Instrument setting
Slide type : 2 channel slide
Autofocused counting : On
Autofocus upon slide insertion : On
Last calibration : 10 Apr 2017 11:45
Calibration value : 0x01FD, 0x03F6

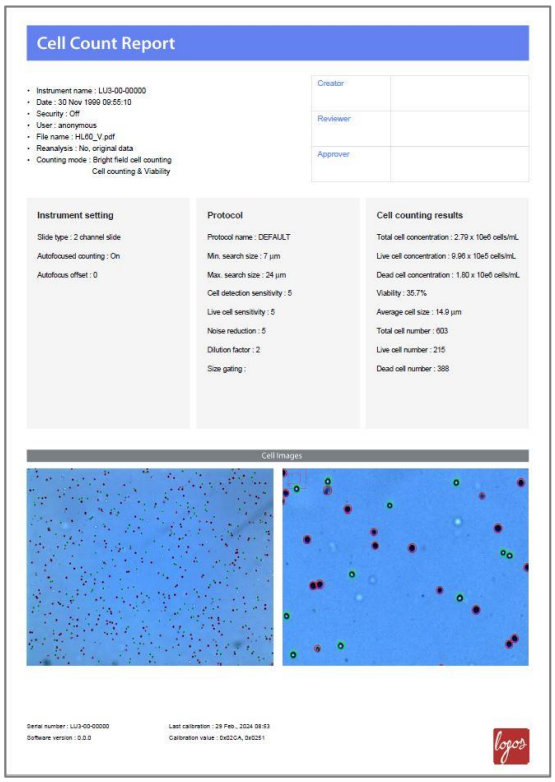
Protocol
Protocol name : DEFAULT
Min. search size : 3 µm
Max. search size : 70
Cell detection sensitivity : 5
Live cell sensitivity : 8
Noise reduction : 5
Dilution factor : 2
Size gating :

Cell count results
[Total cell] : 1.04 x 10e6 cells / mL
[Live cell] : 9.73 x 10e5 cells / mL
[Dead cell] : 6.62 x 10e4 cells / mL
Viability : 93.6 %
Avg. size : 16.5 µm
Total cell number : 251
Live cell number : 235
Dead cell number : 16

==========

SS

HL60.pdf



Cell Count Report

- Instrument name : LU3-00-00000
- Date : 30 Nov 1999 09:55:10
- Security : Off
- User : anonymous
- File name : HL60_V.pdf
- Reanalysis : No, original data
- Counting mode : Bright field cell counting
  Cell counting & Viability

Creator	
Reviewer	
Approver	

Instrument setting

Slide type : 2 channel slide

Autofocused counting : On

Autofocus offset : 0

Protocol

Protocol name : DEFAULT

Min. search size : 7 µm

Max. search size : 24 µm

Cell detection sensitivity : 5

Live cell sensitivity : 5

Noise reduction : 5

Dilution factor : 2

Size gating :

Cell counting results

Total cell concentration : 2.79 x 10e6 cells/mL

Live cell concentration : 0.96 x 10e5 cells/mL

Dead cell concentration : 1.80 x 10e6 cells/mL

Viability : 35.7%

Average cell size : 14.9 µm

Total cell number : 603

Live cell number : 215

Dead cell number : 388

Cell Images

Serial number : LU3-00-00000
Software version : 0.0.0

Last calibration : 29 Feb., 2024 09:53
Calibration value : 0x02CA, 0x0251

Cell Count Report

- Instrument name : LU3-00-00000
- Date : 30 Nov 1999 09:55:10
- Security : Off
- User : anonymous
- File name : HL60_V.pdf
- Reanalysis : No, original data
- Counting mode : Bright field cell counting
  Cell counting & Viability

Creator	
Reviewer	
Approver	

- Cell size histogram expressed by cell number

- Cell size histogram expressed by cell concentration

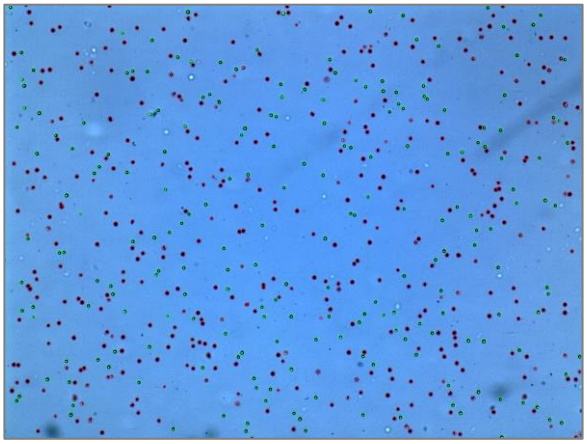
- Cell cluster graph

Serial number : LU3-00-00000
Software version : 0.0.0

Last calibration : 29 Feb., 2024 09:53
Calibration value : 0x02CA, 0x0251

HL60_RAW.tif

HL60_TAG.tif

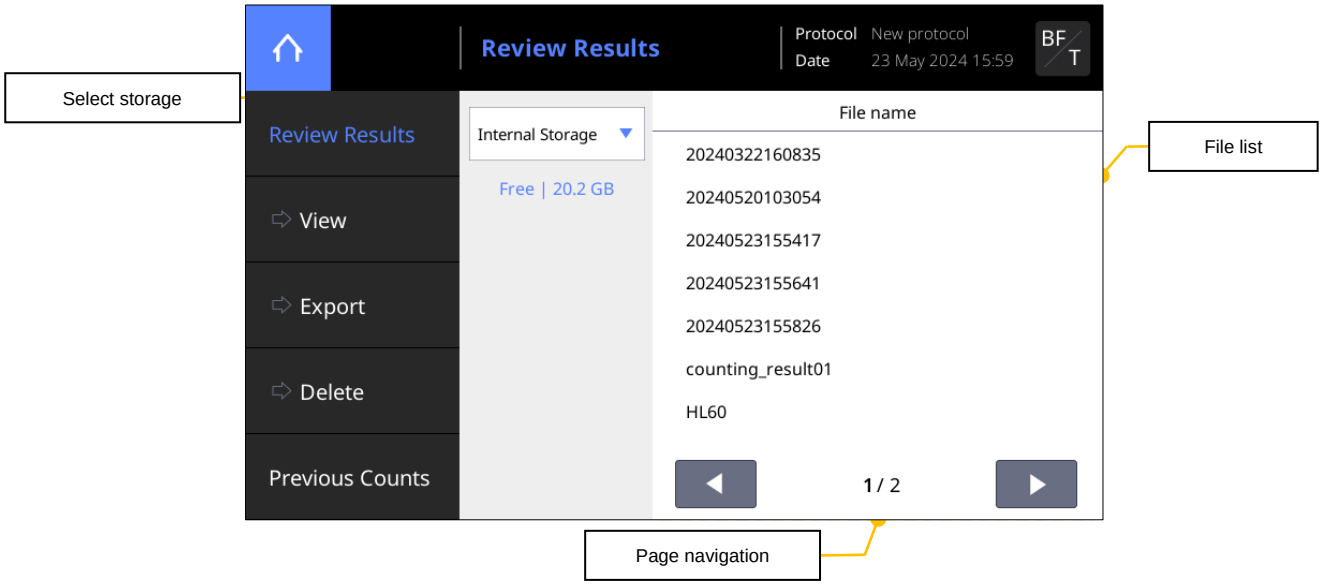


Chapter 5 – Review Previous Results

5.1 Reviewing Data

Select storage

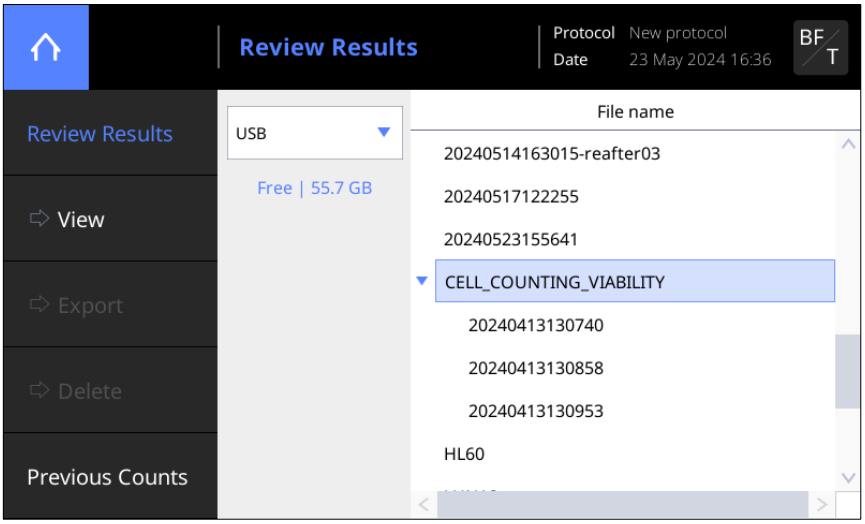
Press **REVIEW** and select **Internal Storage**.



Navigate and open a folder from the internal. Max. 7 folders will be displayed per page. Press arrow button to navigate next page. Press page number to go to the specific page directly.

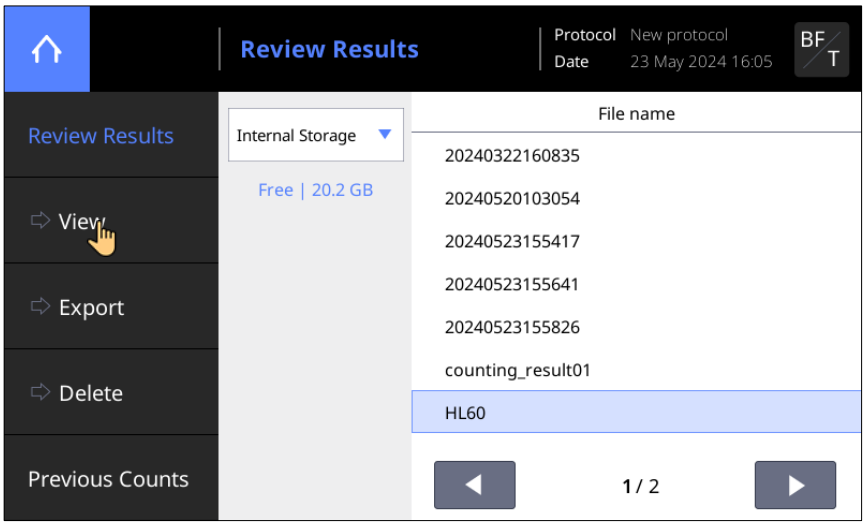
Select USB drive.

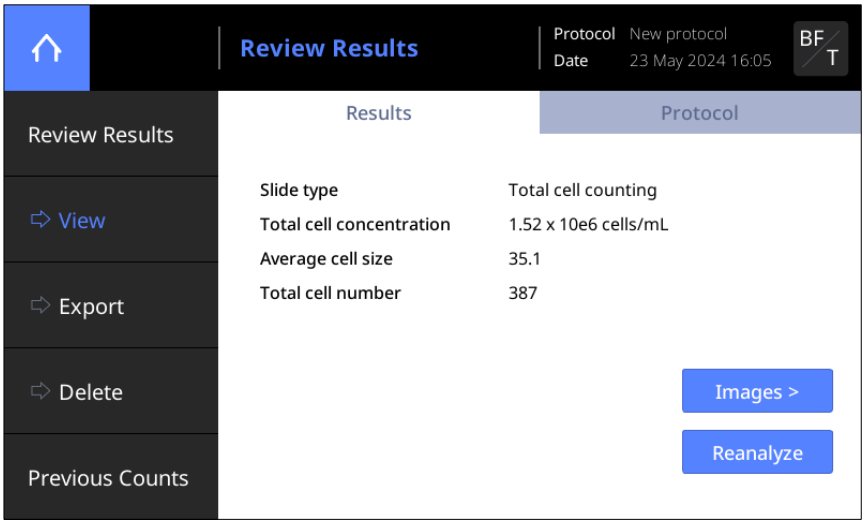
Navigate and open a folder from the USB drive. There is no page movement button in the USB file list. The Export and Delete buttons are disabled.
When multiple USB drives are connected to the equipment, only the first connected drive is activated.



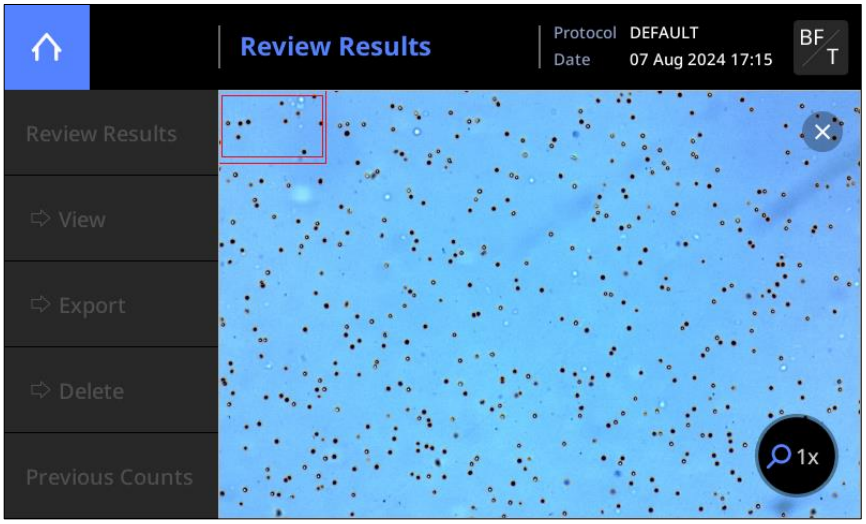
Review Images

After select a folder and press [**View**], Cell counting results will appear.





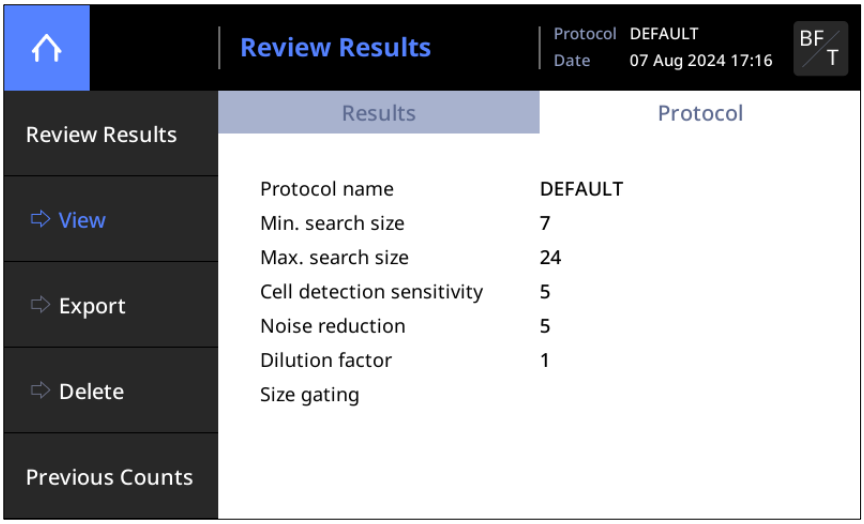
Press [**Images >**] to see image.



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

To transfer files to a USB drive or delete files from the internal drive, press [**X**] button in the top right corner to return to the main Review window

Press the **PROTOCOL** tab to check the protocol used.

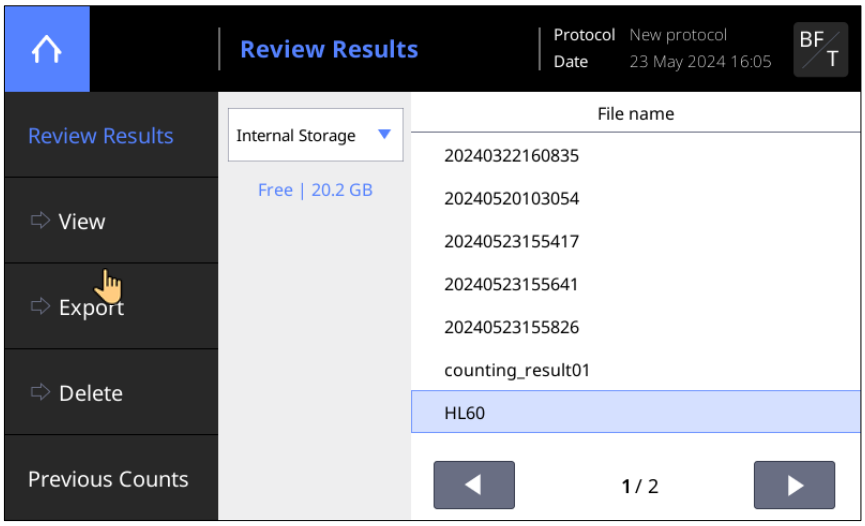


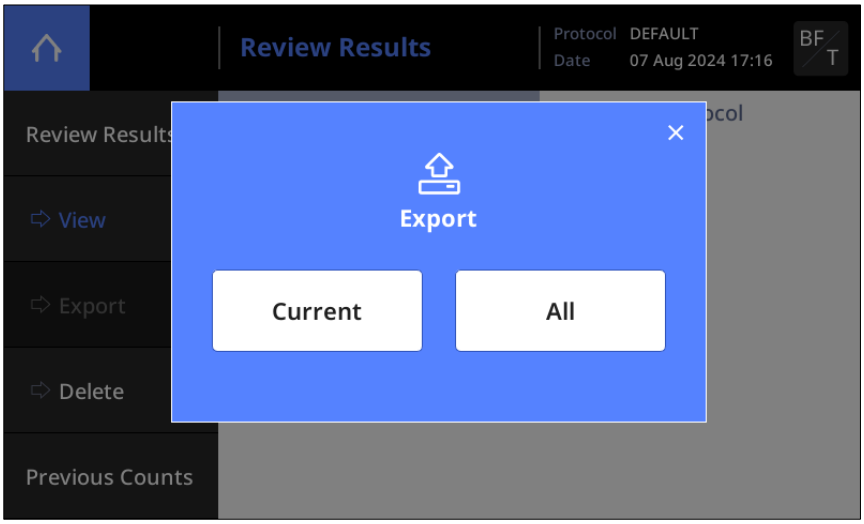
Export data

Data stored in the internal storage can be exported to an external USB drive from the [**Review Results**] tab.

Insert a USB flash drive.

After select a folder and press [**Export**].





An export selection window will appear.

[**Export Current**] : Export only the selected data to the USB flash drive.

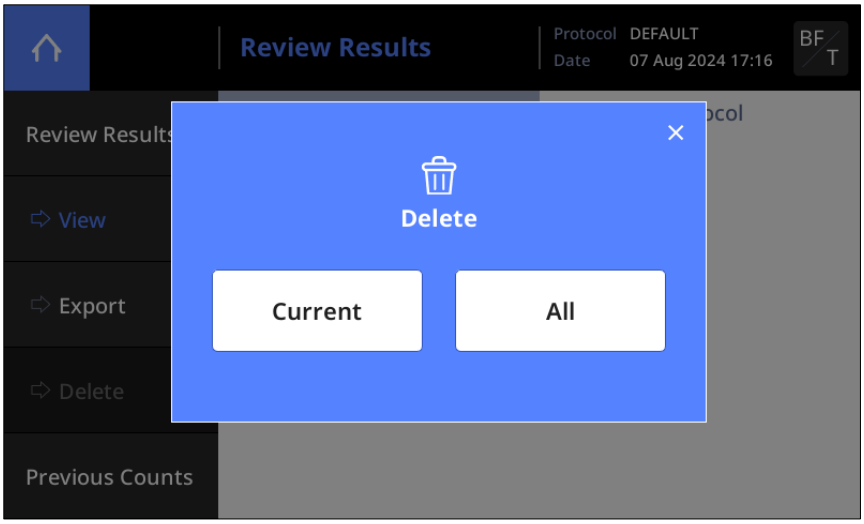
[**Export All**] : Export all counting data from the current count mode to the USB flash drive.

Delete data

Data stored in the internal storage can be deleted using the following step.

Press [**Review Results**] tab.

After select a folder and press [**Delete**].



An delete selection window will appear.

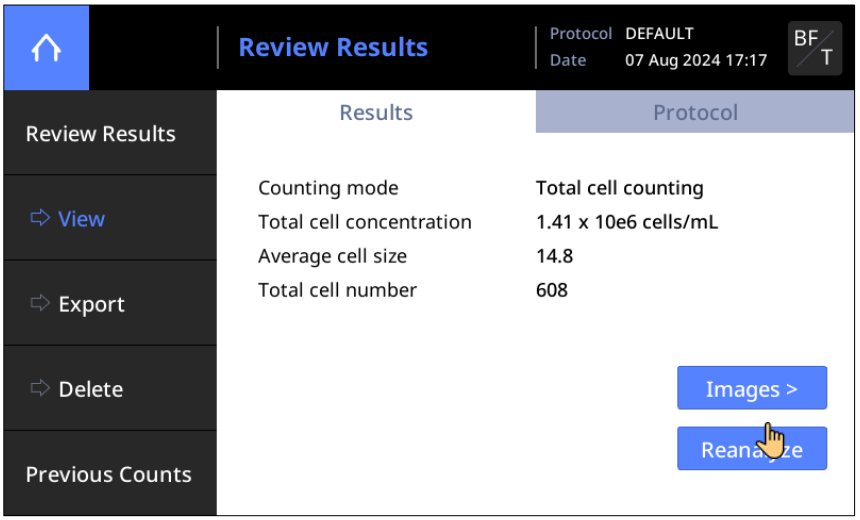
[**Delete Current**] : Delete only the selected counting data.

[**Delete All**] : Delete all counting data in the current counting mode.

Reanalyze

In LUNA-III, you can reanalyze previously acquired cell counting results using different protocols. To use the Reanalyze function, please follow these steps:

Load or create the desired protocol.



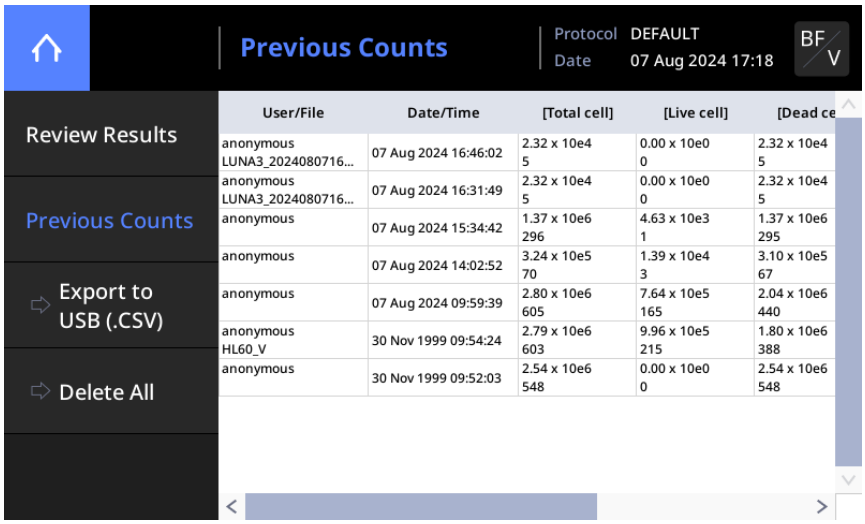
Select a folder to reanalyze from the Internal Storage or USB drive, then press VIEW.

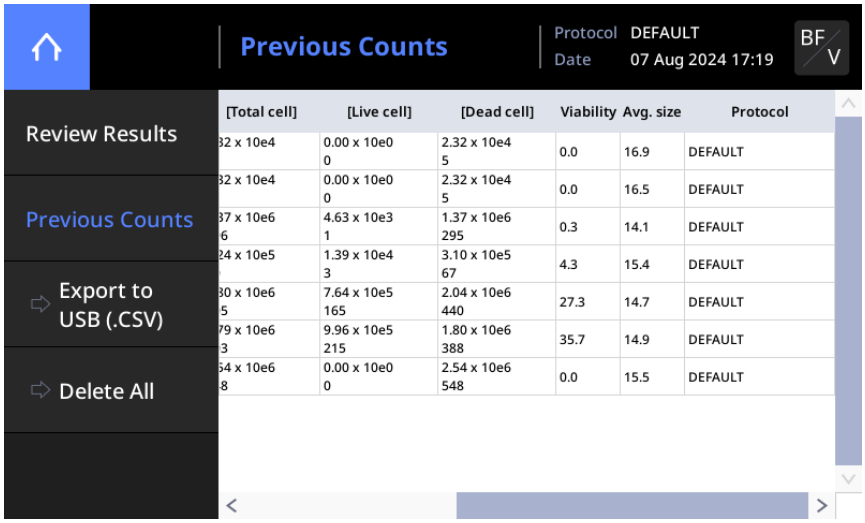
Press [**Reanalyze**].

! **Important !** Reanalysis can only be performed in the same count mode.

Previous Counts

Press [**Previous Counts]** to see a list of previous counts.





[Previous Counts] is a summarized version of the cell counting results previously performed. It includes Name/Date, Total cell concentration, Live cell concentration, Dead cell concentration, Viability, Average size, and Protocol, which are automatically saved to the internal storage.

Press [Export] to export the Previous Counts as a CSV file to the USB drive.

Press [Delete All] to remove Previous Counts. This action will not delete cell counting results, such as PDF reports or cell images.

Chapter 6 – Data Transfer via Network

6.1 Network Sharing

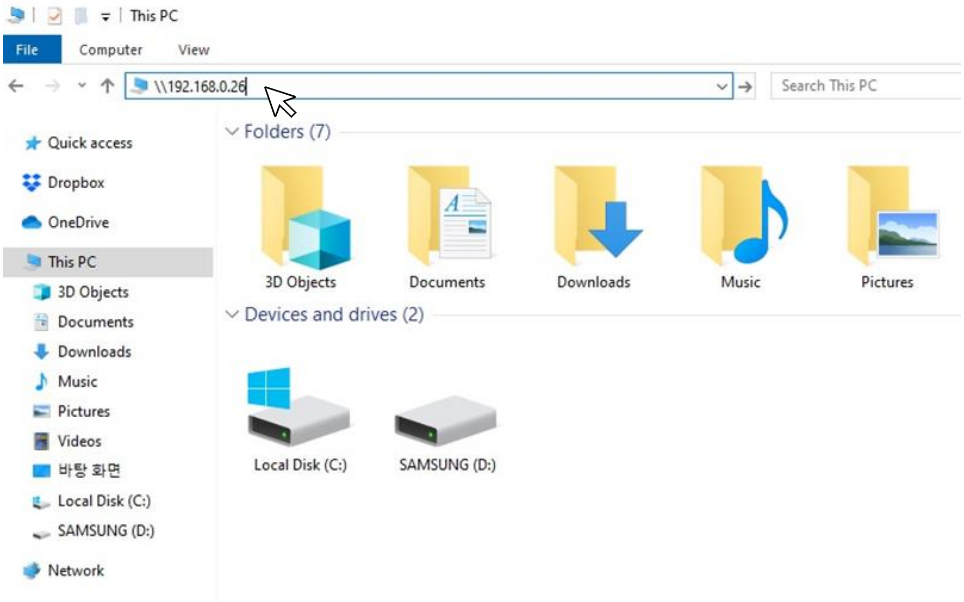
Connected to User PC

Connect the LUNA-III™ to a network. Make note of the LUNA-III™ IP address in the SETTINGS : NETWORK screen. (See Section 2.3 : Settings : Networks)

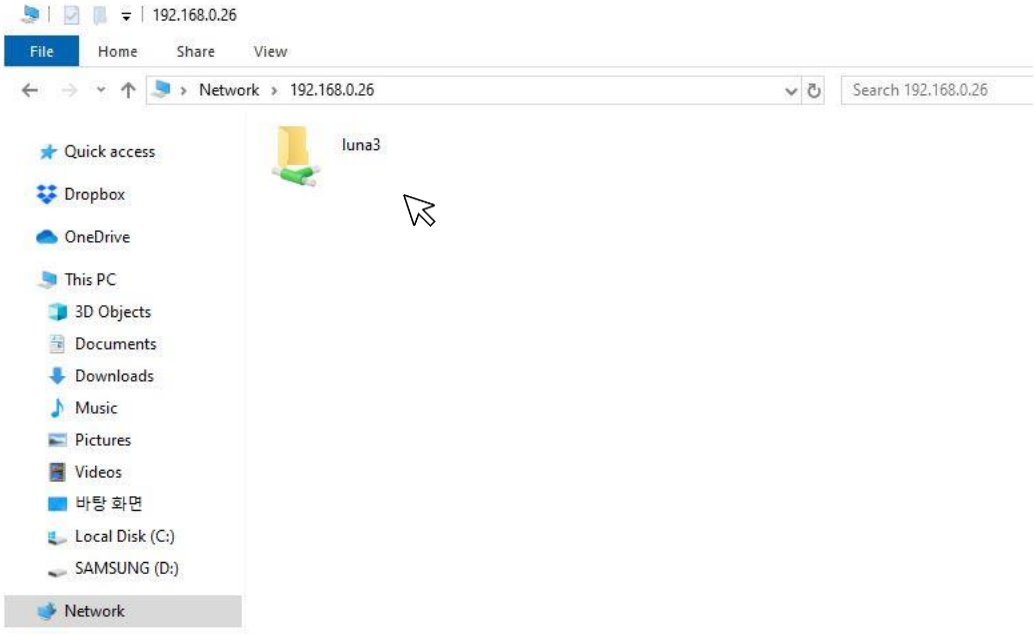
In your Windows PC, open File Explorer (Windows key + E)

Type the IP address connected to the LUNA-III™ in the location directory and press Enter.

e.g. \\192.168.0.26

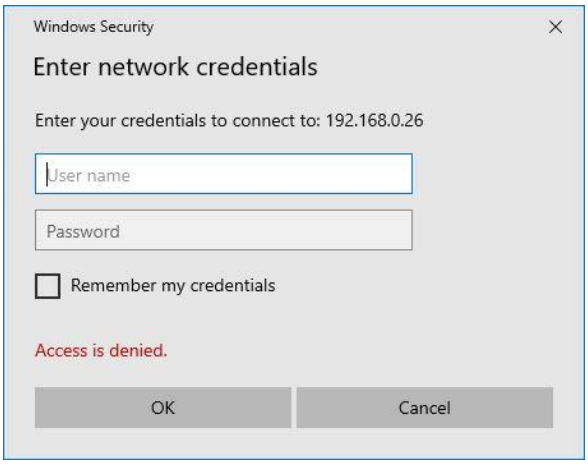


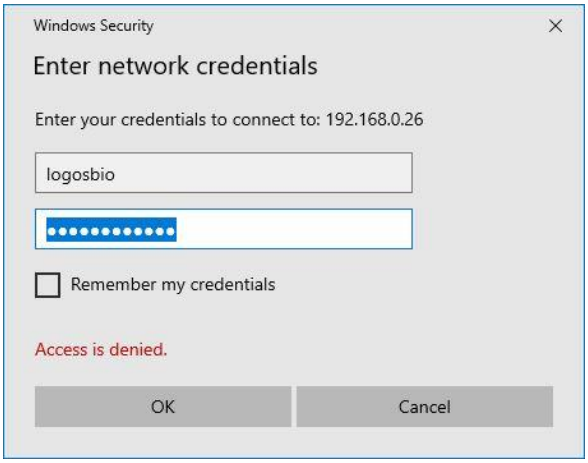
You can double-click and open the **luna-III** folder.



If it is the initial access, it may require a log-in with User name and Password.

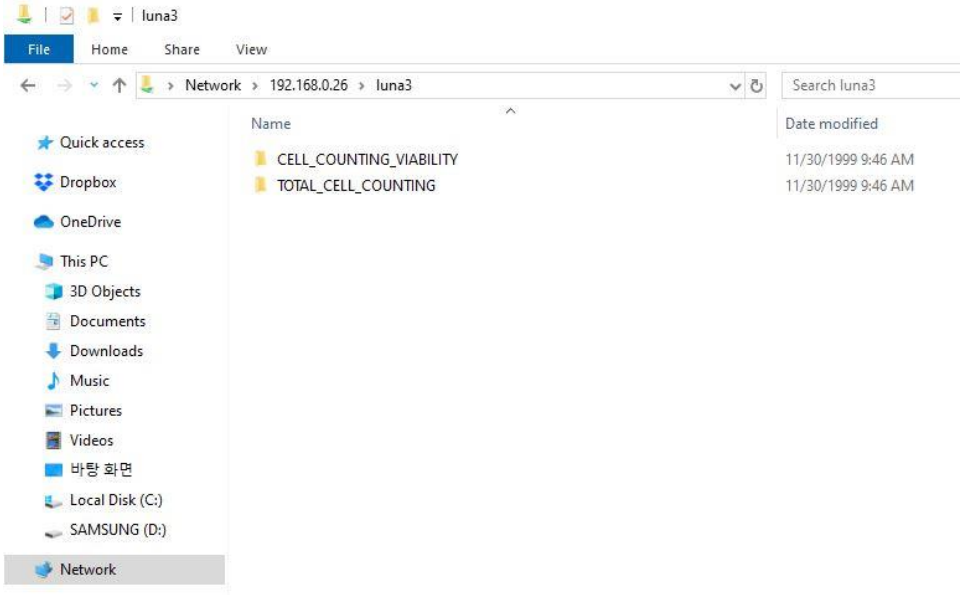
- User name : logosbio
- Password : serial number (Hyphen must be included)



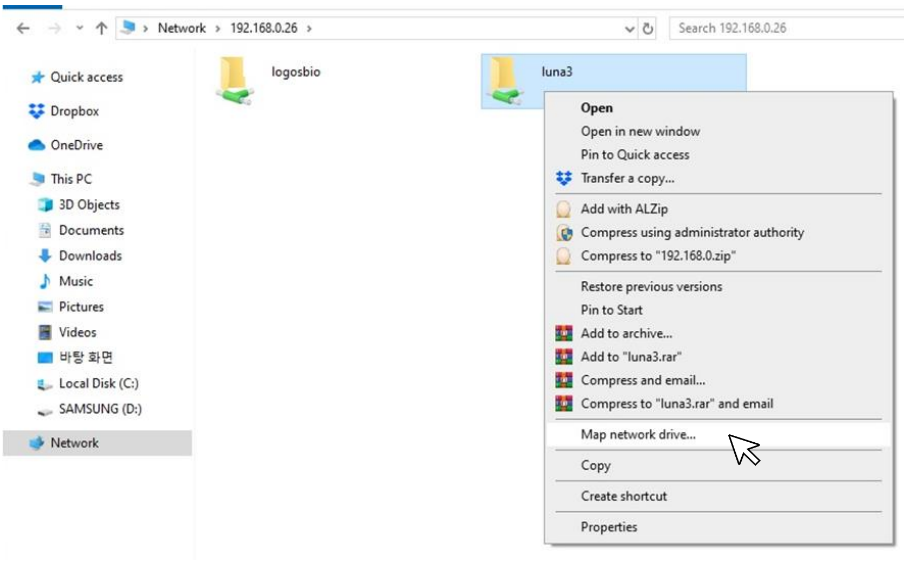


Once logged in, you can transfer data directly from your device.

The data in the folder is not stored in the PC. You may move data from the **luna-III** folder to another drive in the PC.



You can right-click the folder to map the network drive or create shortcut for your convenience.



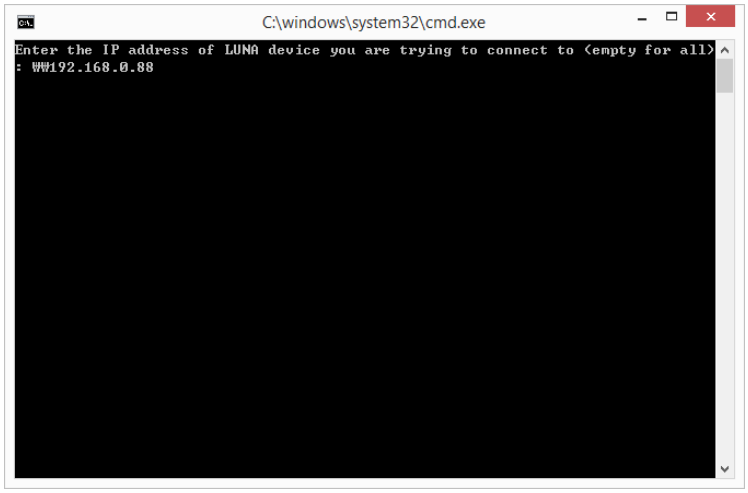
! **Important !** If you cannot access the folder with the message below,

1) Please check if the IP address is correct and the LUNA-III™ is well connected to the internet.

2) If the connection issue still persists, contact Logos Biosystems or your local distributor to receive a script in a Zip file to clean Windows authentication caches.

- Unzip the received file and run the command file.
- Type the IP address and press the Enter key.

  e.g. \\192.168.0.26
- Press any key.
- Try to go through from the beginning.



Chapter 7 – Maintenance and Troubleshooting

7.1 Turning On/Off

To turn the instrument on, push the power button below the touchscreen. (The booting time may take longer during initial operation due to system initialization.)

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-III™ 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 three seconds. If the instrument is frozen due to malfunction, reconnect power cable in the back side of the instrument.

7.2 Cleaning

Turn the LUNA-III™ 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.

7.3 Troubleshooting

Problem	Possible Cause	Solution
Inaccurate cell count	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 x 10^4 -1 x 10^7 cells/mL are optimal for counting. Dilute or concentrate cell suspensions accordingly.
	Improper slide insertion	Make sure that the slide has been properly inserted into the instrument.
	Improper sample loading	Do not over- or under-fill the slide chambers. Carefully load the chambers with 10-12 µL 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 saving	Too many files in the LUNA-III™	Delete or transfer files.
Data transfer	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.
	Incorrect network connection	Make sure LUNA-III and your PC are connected to the same network.
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.
	Trypan Blue Stain was not diluted	Refer to the User Manual and try the background calibration again.
	Trypan Blue Stain precipitation	Please refer to "How to Minimize Trypan Blue Precipitation" on our website (www.logosbio.com) to remove precipitation from the Trypan Blue Stain, or use a different lot of Trypan Blue Stain.
Errors while updating software	Incompatible USB drive	Some USB devices are undetectable or incompatible. Use the USB supplied with the instrument or use a USB 2.0.

	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.

Chapter 8 - Safety Information

8.1 Instrument Safety

General Safety

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 the power source and the instrument. Turn the instrument off before disconnecting the power cord 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-III™ Automated Cell Counter is an electrical laboratory instrument for scientific research use only. It is not a medical, therapeutic, or in vitro diagnostics device.

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	5 - 40°C
Maximum Relative Humidity	20 - 80%
Altitude	≤ 2,000 m
Pollution Degree	2

8.2 Personal Safety

Read all user manuals thoroughly before using the instrument.

Keep all user manuals in a safe and accessible place for future reference.

Wear appropriate personal protective equipment (PPE) when handling reagents and samples to avoid exposure.

When using toxic agents, radioactive materials, or pathogenic microorganisms belonging to WHO Risk Groups 2-4, follow national laws and regulations for biosafety level requirements.

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

8.3 Instrument Symbols

Symbol	Description
	Protective earth (ground) terminal
	Waste Electrical and Electronic Equipment (WEEE). Do not dispose of this product as unsorted municipal waste. Follow local waste ordinances for proper disposal provisions to reduce the environmental impact of WEEE.

8.4 Safety Standards

European Standards

Symbol	Description
CE	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 Logos Biosystems.

Korean Standards

Symbol	Description
	The KC certification mark indicates that this instrument conforms with Korea's product safety requirements for electrical and electronic equipment and components for which this marking is required.

United States Standards

Type	Description
FCC Part 15	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.

Chapter 9 - Ordering Information

Cat #	Product	Quantity
L60001	LUNA-III™ Automated Cell Counter	1 unit
L60002	LUNA-III™ Automated Cell Counter sustainable package	1 unit
L12001	LUNA™ Cell Counting Slides, 50 slides	1 box
L12002	LUNA™ Cell Counting Slides, 500 slides	10 boxes
L12003	LUNA™ Cell Counting Slides, 1,000 slides	20 boxes
L12011	LUNA™ Reusable Slide	1 unit
L12012	LUNA™ Reusable Slides (2pack)	2 units
L12014	LUNA™ Reusable Slide Coverslips	10 units
T13001	Trypan Blue Stain, 0.4%	2 x 1 mL
T13011	Trypan Blue Stain, 0.4%, Sterile-filtered	2 x 1 mL
L13002	Erythrosin B Stain	2 x 1 mL
B13001	LUNA™ Standard Beads	2 x 1 mL
P17001	LUNA™ Printer II	1 unit
U10005	USB Drive, 16 GB	1 unit
L72041	Cell Counter Validation Slide-BF II	1 unit
L64003	LUNA-III™ IQ/OQ Protocol	1 copy

Chapter 10 - Purchaser Notification

10.1 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 bounded 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.

Aligned Genetics, 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.

10.2 Instrument Warranty

Aligned Genetics, 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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Aligned Genetics, Inc.

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