LUNA II™

Automated Cell Counter

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IMPORTANT

The LUNA-II™ Automated Cell Counter is a Laboratory Electrical Instrument for Scientific Research Use Only, BUT NOT A MEDICAL or THERAPEUTIC or IN VITRO DIAGNOTICS DEVICE.

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Safety Information

For best results with the LUNA-II™ Automated Cell Counter, follow the instructions below in addition to the general precautions for using electrical instruments.

1. Be careful to avoid electric shock while operating the instrument. Do not touch it and other components with wet hands. Do not place it in a humid environment such as an incubator. For operating environment, see page 9.
2. Trypan blue stain is known as a hazardous material. While handling the solution, always wear proper personal protective equipment (PPE) to avoid exposure.
3. Before use, make sure that the input voltage is compatible with the instrument's power supply voltage.
4. For optimal operation, place the instrument on a flat surface and avoid any vibration.
5. Turn on the instrument only after connecting both ends of its power cord to the wall outlet as well as the instrument. Always turn off the instrument before disconnecting the power cord and/or moving the instrument.
6. Ensure that the power cord is firmly plugged into the power inlet, the wall outlet and AC adapter.
7. When the instrument is operating for a long time, its temperature can become too high. Please be careful that the instrument's temperature does not become too high during long and continuous operation times. When operating, leave enough space around the instrument so there is enough room for air circulation and cooling.
8. Do not disassemble the instrument in any event. If the instrument is out of order or dropped or broken, please contact a service person. *Disassembling the instrument invalidates its warranty.*
9. Use only authorized components (adaptor, power cord, and USB drive).
10. If the instrument emits smoke, disconnect the power cord immediately from the wall outlet and contact a service person.
11. Used counting slides must be disposed as biohazard wastes.

<Symbols used in this User Manual>

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 ecologically friendly manner. Follow 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)

General Guidelines for Using the LUNA-II™ Automated Cell Counter

In order to achieve the best results with the LUNA-II™ Automated Cell Counter, follow the instructions below carefully.

1. The instrument must be operated in compliance with the operating environment described on page 9. In particular, the temperature and humidity conditions are important.
2. Samples must be handled in an appropriate way, depending on user's requirements.
3. Hold the Luna™ Cell Counting Slide by the edges to avoid touching its optical surface. Make sure that no damage or contamination occurs on the optical surfaces of the slide.
4. After mixing the cell sample with trypan blue stain, perform cell counting as soon as possible, within 3 minutes for accurate cell viability measurement. If needed, count your sample at least 2 times (duplicate readings) and take an average.
5. Since the LUNA-II™ Automated Cell Counter is calibrated before shipping, you do not need to re-calibrate before use. However, if re-calibration is needed, please refer to Section 5.3 Calibrating the Counter.
6. Do not touch trypan blue solution with bare hands as it is a hazardous chemical. After using the counting slides, dispose of it as hazardous wastes. Do not reuse the slides.

Environment Conditions

Operating Power	100 – 240 VAC, 1.2 A
Frequency	50/60 Hz
Electrical Input	12 VDC, 3.3 A
Installation Site	Indoor use only
Operating Temperature	10 – 35℃
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 a small, fast, and affordable image-based cell counting device that automatically counts various kinds of cells for research purpose.

The LUNA-II™ Automated Cell Counter helps measure the number, as well as viability of cells (live, dead, total cells) with sophisticated optical components and advanced image analysis algorithms. Due to several innovations introduced by Logos Biosystems, LUNA-II™ provides a state of the art cell counting device and eliminates the tedium and subjectivity of manual cell counting.

The LUNA-II™ Automated Cell Counter can be used in a very simple procedure. First, mix 10 μl of the cell sample with 10 μl of trypan blue stain. Second, load the cell suspension into the Luna™ Cell Counting Slide. Third, insert the slide into the slide port of the instrument and chose [Autofocused Counting] or adjust the focus by clicking arrows to get an appropriate cell image. Last, press the [Count] button and then the results of cell count and viability will be displayed on the screen. The counting image can be downloaded onto a USB drive in TIF format for review and/or record keeping.

The LUNA-II™ Automated Cell Counter provides key data as below:

- Number of live and dead cells/ml
- Number of total cells/ml
- Viability percentage (% live cells to total cells)
- Cell images (showing live cells as green circles and dead cells as red circles)
- Histograms of cell size distributions

The Luna™ Cell Counting Slide is disposable and specifically designed for the LUNA-II™ Automated Cell Counter. Each counting slide has 2 chambers, labeled as A and B, respectively, so that one slide can be used for the same sample reading in duplicate or for 2 different samples if preferred.

1.2 Key Features

Key features	Description
Small footprint	Compact size with light weight saves space and is suitable for either laboratory table or biosafety cabinet.
Accuracy & precision	With sophisticated optical components and counting algorithm, LUNA-II™ provides optimized and reproducible results every time.
Autofocusing	Advanced liquid lens technology provides fast autofocusing without mechanical moving. Autofocusing enables reliable cell counting and excludes human error.
Easy-to-operate user interface	The intuitive user interface based on a touch screen enables simple and easy operation.
Shortest time-to-results	Results for most cell lines are available within 15 (without autofocusing) or 22 (with autofocusing) seconds after pressing the [Count].
Built-in printer (Optional)	An integrated thermal printer provides concise Cell Count Report facilitating record keeping.
Innovative counting slide	LUNA-II™ adopts an innovative counting slide made with “T-BOND” technology without using hazardous organic solvents.
Cell concentration & viability range	Measurements can be made for cells at concentrations ranging from 5 x 10^4 to 1 x 10^7 cells/ml and for cells within the 3 – 60 μm diameter range.
Easy-to-use calculator	No external calculator is needed to calculate subsequent dilution.
Setup & maintenance	Just plug in and it is ready for use, with virtually no maintenance costs.
Counting image acquisition	The captured image of cells can be downloaded onto a USB drive in TIFF (Tag Image File Format) for review or record keeping.
Individual protocol	Different protocols (up to 300 protocols) can be saved with personalized parameters.
Documentation	LUNA-II™ provides a PDF (Portable Document Format) report which includes File name, Date, Cell count results, Cell images, and histograms.

1.3 Product Contents

The product package of the LUNA-II™ Automated Cell Counter contains the following components.

Component	Quantity
LUNA-II™ Automated Cell Counter (with or without printer)	1
Power cord (including an adapter)	1
Luna™ Cell Counting Slides	1 box (50 slides for 100 counts)
Trypan Blue Stain (0.4 %)	2 x 1 ml
Luna™ USB drive	1

After receiving the product package, please immediately unpack it and check the components listed above to ensure that all parts are included and no damage has been 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.

Note: The LUNA-II™ Automated Cell Counter is only for research purpose, not for human or animal therapeutic or diagnostic use.

1.4 Product Specifications

1.4.1. LUNA-II™ Automated Cell Counter specifications

Instrument Type	Benchtop cell counter
Dimensions (WxDxH)	16 x 18 x 28 cm (6.3 x 7.0 x 11.0 inch)
Weight	1.6 kg (3.5 lb) without the external power adaptor
Cell Concentration Range	5 x 10^4 – 1 x 10^7 cells/ml
Cell Diameter Range	3 – 60 μm (optimal 8-30 μm)
Cell Viability Range	0 – 100%
Image Resolution	5 mega pixels (5 MP)
Image Type	TIF format (Optimized for LUNA-II™ only)
Software	LUNA-II™ software (www.logosbio.com)
Documentation	PDF report
Processing Time	Nominal time for cell counting is less than 10 (without autofocusing) or 15 (with autofocusing) seconds at ~1 x 10^6 cell/ml concentration. (Processing time may vary by cell type and concentration)

1.4.2 Luna™ Cell Counting Slide specifications

Material	Polystyrene
Dimensions (WxDxH)	25 x 75x 2.4 mm
Chamber Depth	100 μm
Chamber Volume	10 μl

Note: One Luna™ USB Drive (4 Gigabytes) and 0.4% Trypan Blue Stain solution (2 x 1 ml) are included in the starter package.

1.5 Product Description

1.5.1 Front view of the LUNA-II™ Automated Cell Counter

The front view of the LUNA-II™ Automated Cell Counter shows various parts as shown below.

- Wide touchscreen located in the upper front of the instruments contains buttons for all functions needed to operate the instrument and displays data acquired.
- Power button is used to turn on the instrument.
- Counting slide port is used to insert the Luna™ Cell Counting Slide containing sample to analyze.
- The front USB port provides easy-to-access data retrieving to transfer.

1.5.2 Rear view of the LUNA-II™ Automated Cell Counter

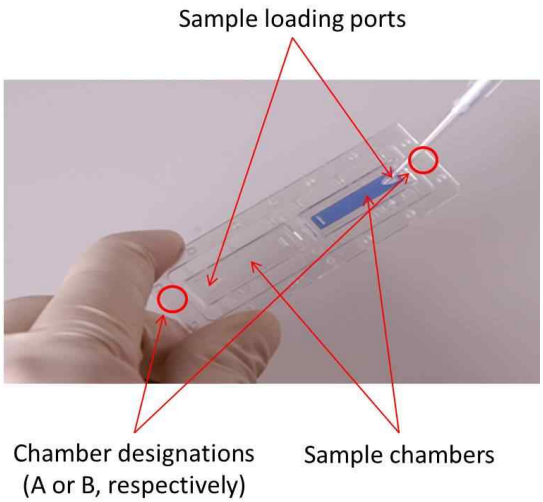
The rear view of the LUNA-II™ Automated Cell Counter shows two additional USB ports and a power inlet to connect the instrument to an electrical outlet with the power cord and plug which are provided in the product package. Be sure to check the electrical outlet configuration in your country.

1.5.3 Right side view of the LUNA-II™ Automated Cell Counter

The right side view of the LUNA-II™ Automated Cell Counter shows a built-in printer (L40001 model only) which allows rapid printing the cell counting results.

1.5.5 Luna™ Cell Counting Slide

The Luna™ Cell Counting Slide is a plastic disposable cell counting slide consists of 2 chambers, labeled as A or B, that can be used for the same sample as duplicate or for 2 different samples. The depth of the counting chamber is 100 μm. The volume of cells counted is about 0.5 μl, almost the same as five (1 mm x 1 mm) squares in a standard hemocytometer.



Chapter 2 – Setting up

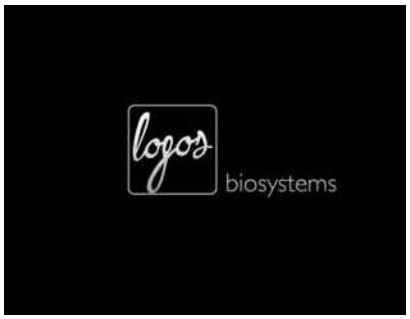
2.1 Installation

Upon receiving the product package, unpack it carefully and ensure that every component is included and no damage has been occurred.

Place the LUNA-II™ Automated Cell Counter on a flat and stable surface.

Insert one end of the power cord into the instrument and plug the other end of the power cord into an electrical outlet after checking the outlet configuration in your local area.

Turn on the instrument using the power button located on the front of the instrument. Initializing screen after company logo will be displayed a few seconds as below.



Company logo

Initializing screen of the LUNA-II™ Automated Cell Counter. In the initializing screen, the current version of LUNA-II™ OS can be identified in the right lower corner. Initializing screen will be followed by LOADING sign as below.

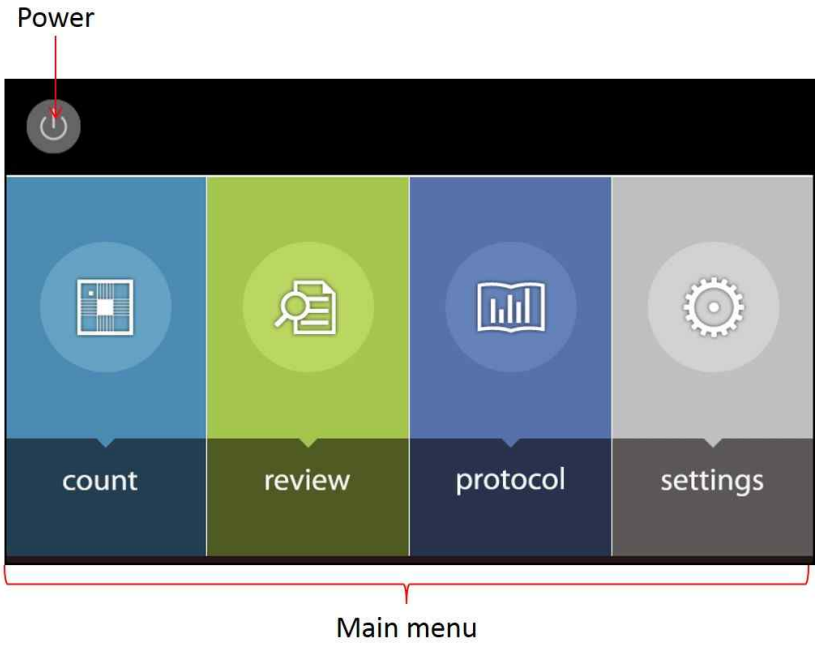
LOADING sign of the LUNA-II™ Automated Cell Counter.

The Start-Up screen will be displayed on the touchscreen as shown below in 2.2.

See Section 7.1 for Turn on and Turn off the instrument.

2.2 Start-Up Screen

The Start-Up screen will show 5 compartments; Power icon, count, review, protocol, and settings.



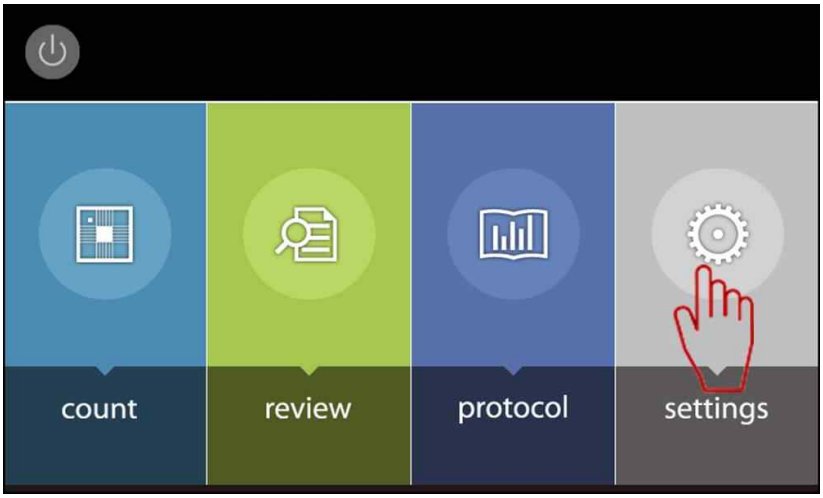
The Power icon in the Start-Up screen can be used to turn off the instrument. See Section 7.1. for further information to turn on/off the instrument.

2.3 Settings

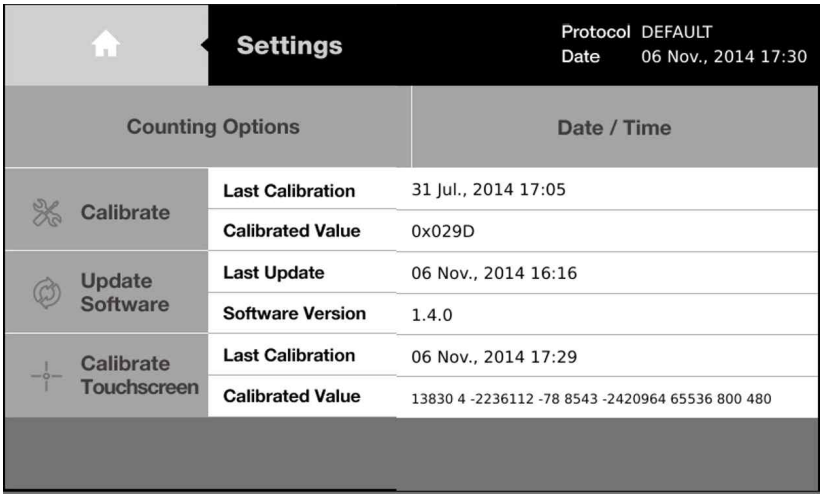
Generally, there is no need to change the settings of the instrument since they are preset at the time of manufacture.

If users need to reset the date, time, or other options, users can adjust or change the options/parameters in the “Settings” menu described below.

Press [settings] located on the right of the Start-Up screen as below.



Then, the following Settings screen will be displayed.



In the Settings screen, users can see the followings:

- Home image: by Pressing Home image, you can go to the Start-Up screen.
- Current protocol and date in the upper right corner.
- Date and values of last calibration.
- Date and software version of latest software update.

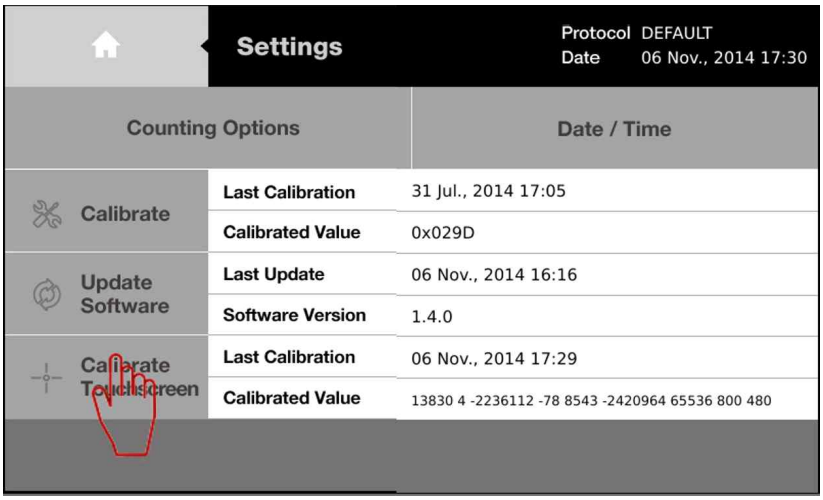
The Settings menu allows you to perform the followings:

- Change options for cell counting by pressing [Counting Options].
- Set the date and/or time by pressing [Date / Time].
- Re-calibrate the instrument by pressing [Calibrate].
- Update the software by pressing [Update Software].
- Calibrate the touchscreen by pressing [Calibrate Touchscreen].

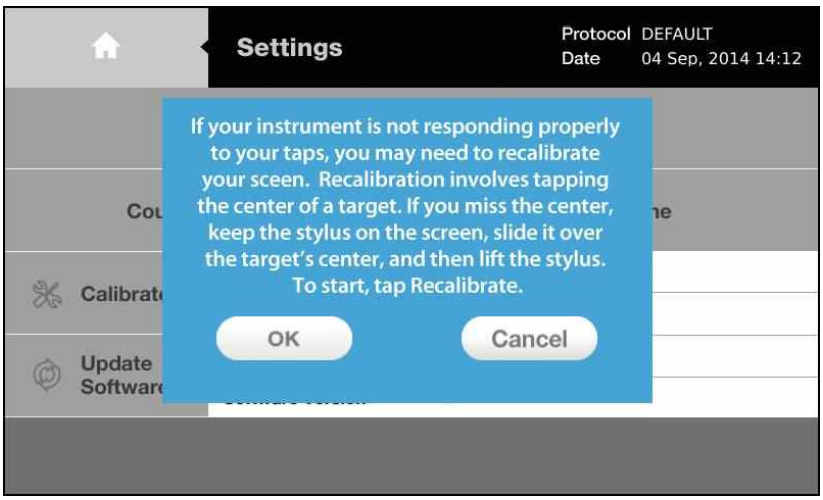
2.4 Calibrating the Touchscreen (Calibrate Touchscreen)

This function can be used when the response of touchscreen is abnormal.

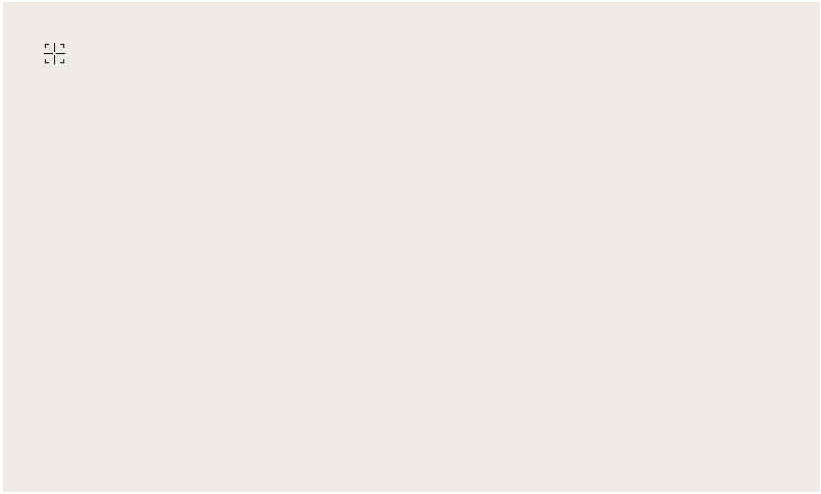
On the Settings screen, press [Calibrate Touchscreen] as below.



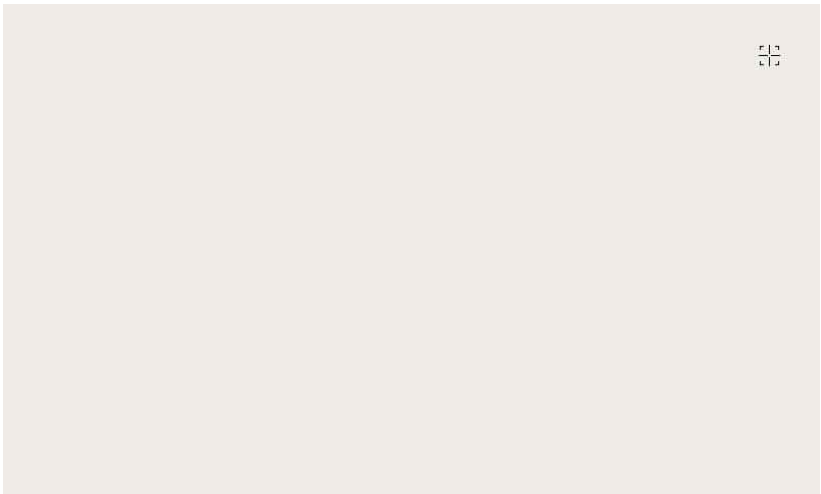
Now, users can see the following screen. If users want to calibrate the touchscreen, press [OK]. If not, press [Cancel].



Now, small cross mark will be appeared in the upper left corner in the grey screen as below.



Press the cross mark, then the cross mark will move to the upper right corner as below.



Press the cross mark again. The mark will move to lower right corner of screen. Press it again. The cross mark will further move to lower left corner and center of screen after each touch. After touch the cross mark in the center, the following sign will be displayed in the center of screen.

After a few seconds, the Settings screen will be re-appeared spontaneously.

Press the Home image to go to the Start-Up screen.

2.5 Changing Options for Cell Counting (Counting Options)

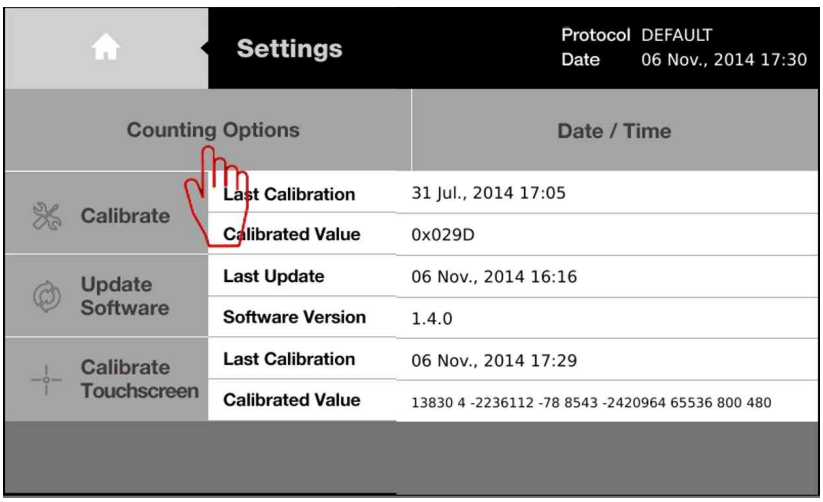
The LUNA-II™ Automated Cell Counter provides two options for cell counting: “With Trypan Blue” or “Without Trypan Blue”. This function provides inter-change of these options.

Option	Description
With Trypan Blue*	This option can be used for regular bright field counting, when cell samples are mixed with 0.4% trypan blue stain in a 1:1 ratio. This option can generate cell viability data. **For cell counting with this option, the dilution factor in the Protocol should be set to value “2”.****
Without Trypan Blue	When samples do not contain trypan blue stain, turn on this option and follow the directions in the message boxes. **For cell counting with this option, please make sure that the dilution factor in the Protocol should be set to value “1”.****

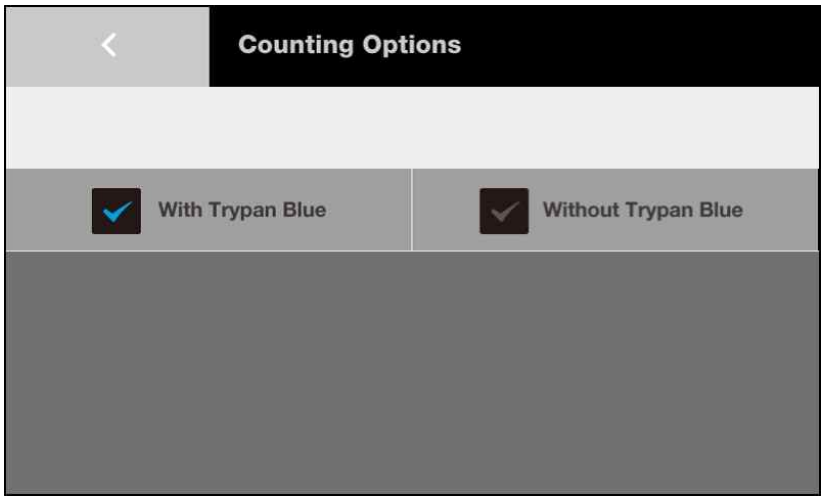
*The cell counting parameters of LUNA-II™ are optimized with the use of trypan blue. Low contrast from no use of trypan blue stain may cause abnormal results.

**The “Dilution Factor” in the “Protocol” is not changed automatically. After changing the “Counting Options”, the “Dilution Factor” should be changed manually. Improper use of the Dilution Factor causes incorrect calculation of cell concentrations.

On the Settings screen, press [Counting Options].

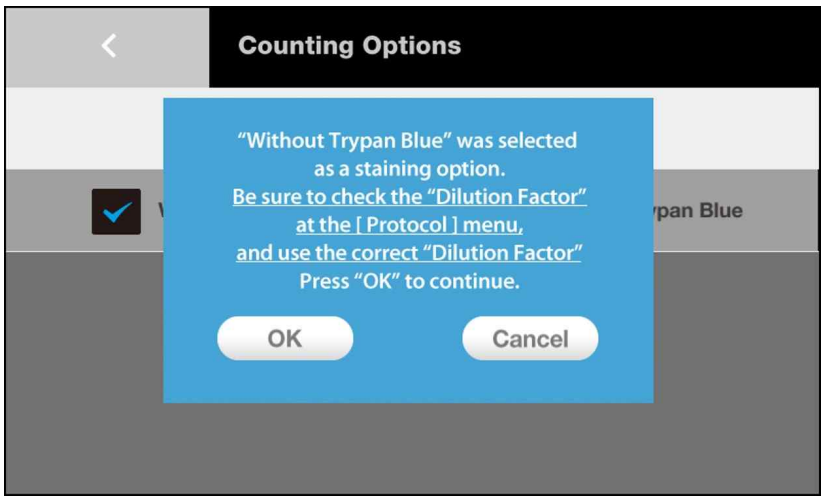


In the Counting Options screen, current option will be marked as blue √ mark as below.

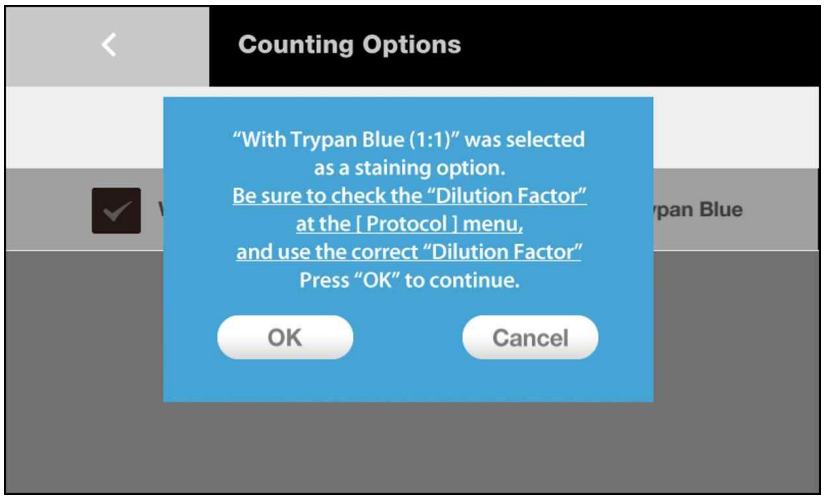


The counting option can be easily changed by pressing un-selected option.

If [Without Trypan Blue] is chosen, the following screen will be displayed.



If [With Trypan Blue] is chosen, the following sign will be displayed.



The Counting Option is simply changed by pressing [OK]. Otherwise press [Cancel] to return the previous option.

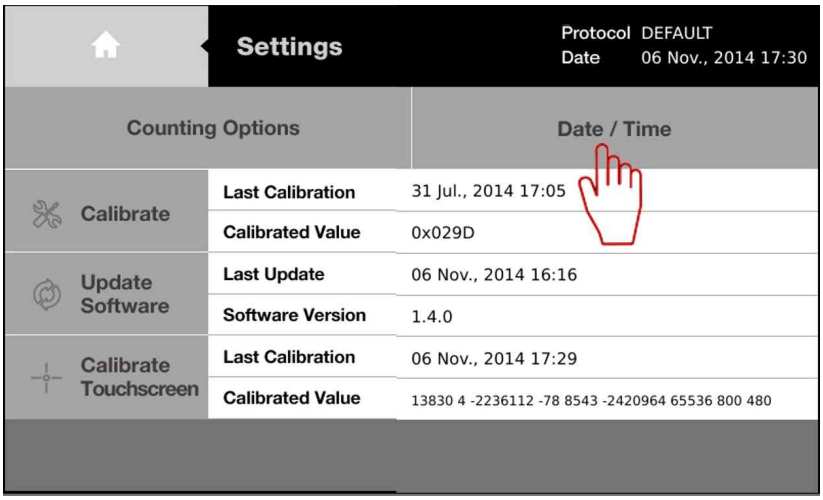
Press [<] in the upper left corner to move to the Settings screen.

2.6 Setting the Date and Time

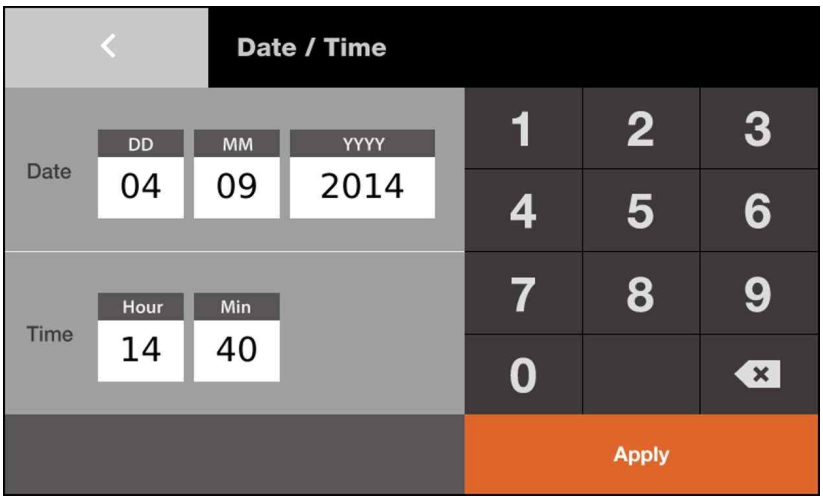
The LUNA-II™ Automated Cell Counter provides current date and time for record keeping. Users may want to change the date and time, since factory setting is adjusted to the local time of Korean.

Once the date and time are set, no additional setting is required for routine laboratory use.

On the Settings screen, press [Date / Time] as below.



The following screen will be displayed.



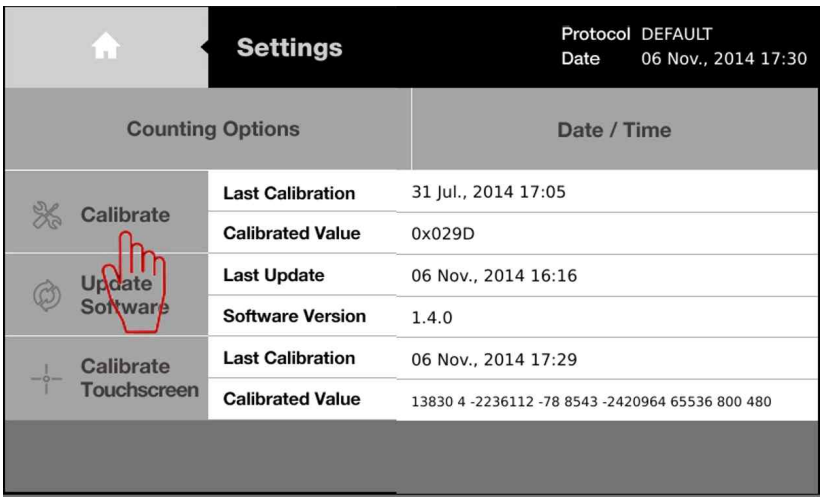
Press desired field to erase the current number. Put number by pressing numbers on the right panel and press [Apply] to save changes.

Press [<] in the upper left corner to move to the Settings screen.

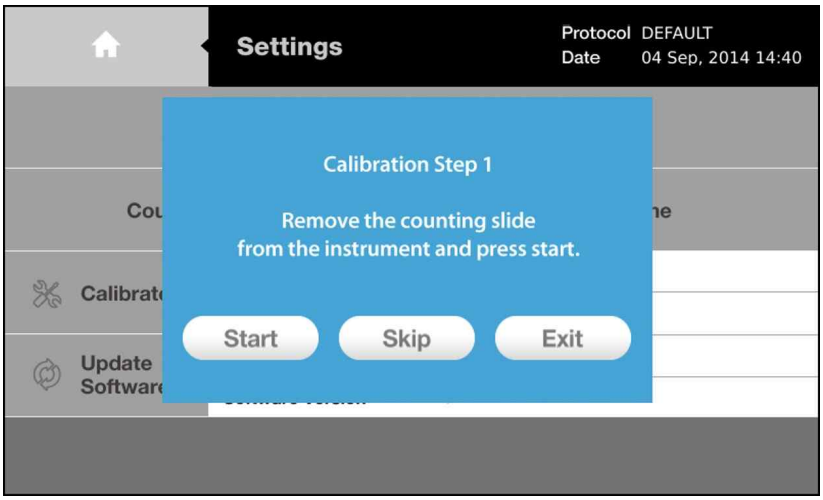
2.7 Calibrating LUNA-II™ (Calibrate)

Background calibration is a prerequisite for successful detection of cells. The LUNA-II™ Automated Cell Counter provides easy-to-use automatic self-calibration of background.

On the Settings screen, press [Calibrate] as below.

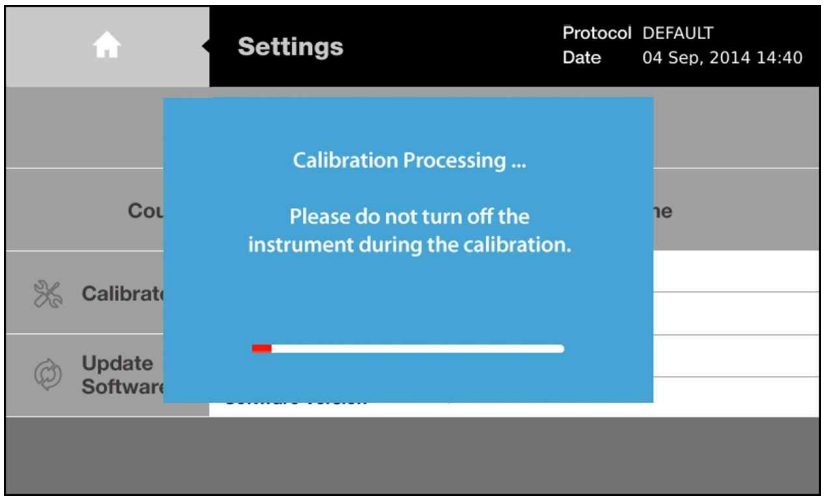


The following screen will be displayed.



For the Calibration Step 1, the counting slide port should be emptied. As directed on the screen, remove the counting slide from the counting slide port of the instrument. Press [Start].

Following screen will be displayed with increasing red bar over the time.

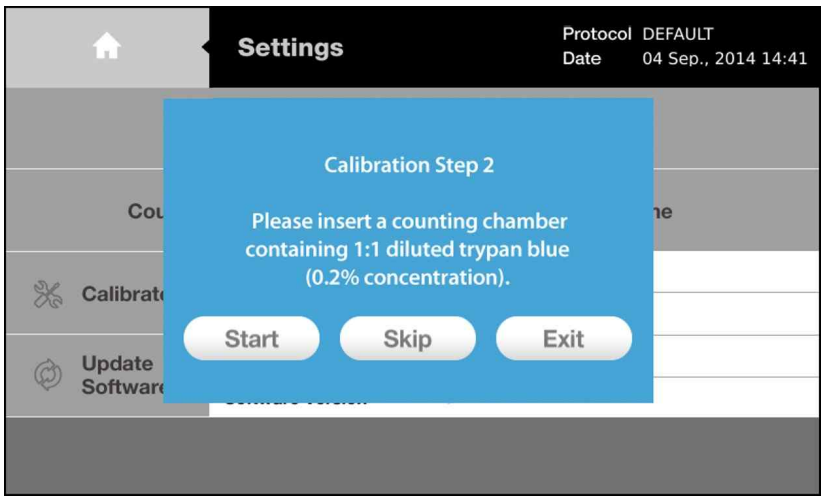


The Calibration Step 1 will take a few seconds. Do not turn off the instrument.

During the process, prepare diluted trypan blue solution by mixing 0.4% trypan blue stain with equal volume of either distilled water, phosphate buffered saline (PBS), or plain medium.

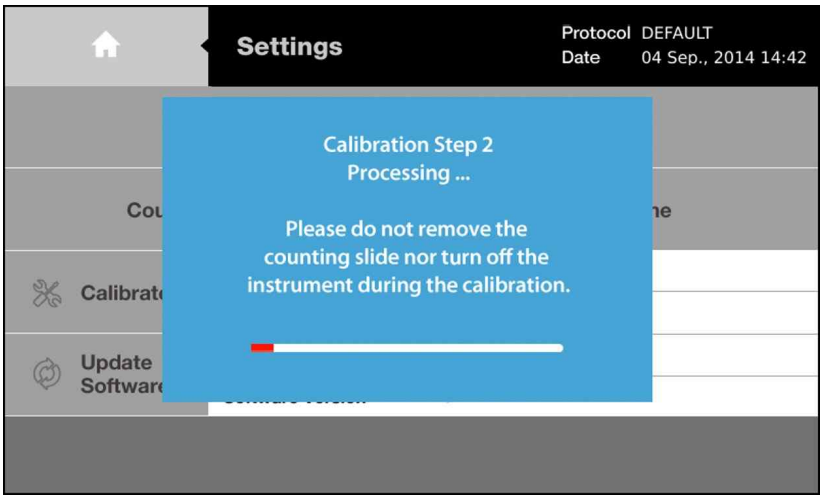
Put 10 μl of diluted trypan blue solution into the chamber of new cell counting slide (see Section 4.3).

After completion of the Calibration Step 1, following screen will be displayed.

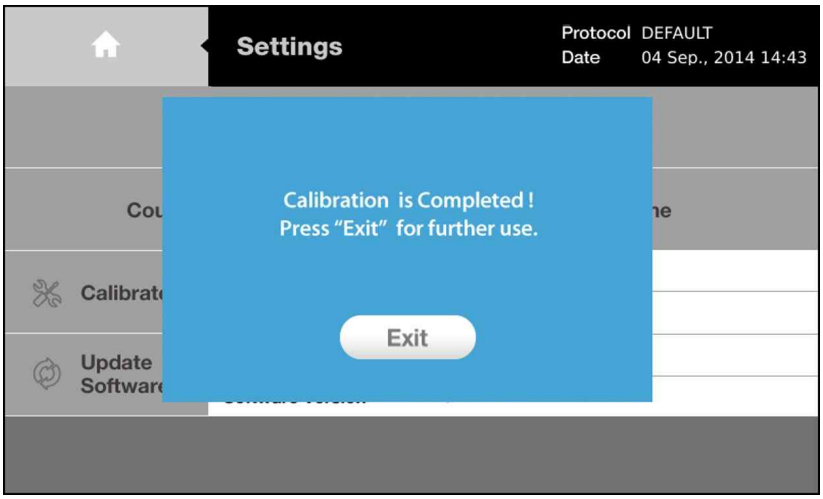


Now, place the cell counting slide containing diluted trypan blue solution into the counting slide port. Be sure the chamber containing the trypan blue solution to be placed inside the instrument. Do not turn the cell counting slide upside-down.

Press [Start] to initiate the Calibration Step 2. Following screen will be displayed with increasing red bar over the time. Do not turn off the instrument.

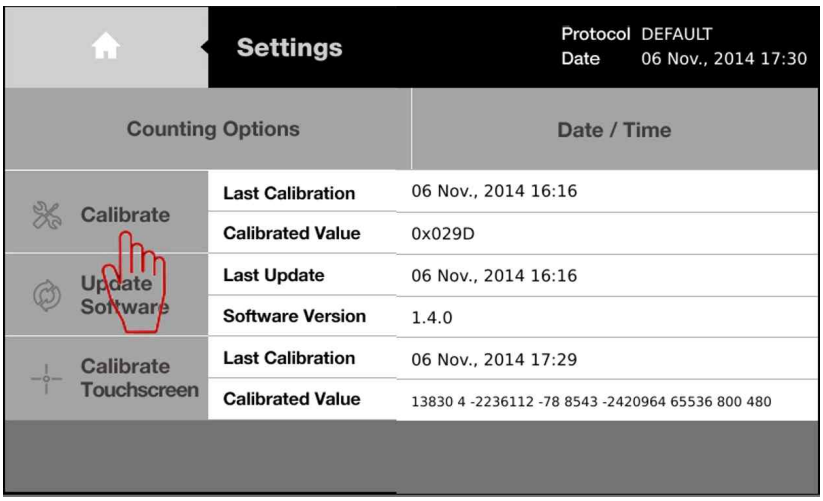


After completing calibration, the instrument will display following sign on the screen.



Press [Exit] to move to the Settings screen.

Now, the date of last calibration is changed (Compare to the Settings screen in page 26).



2.8 Updating the Software (Update Software)

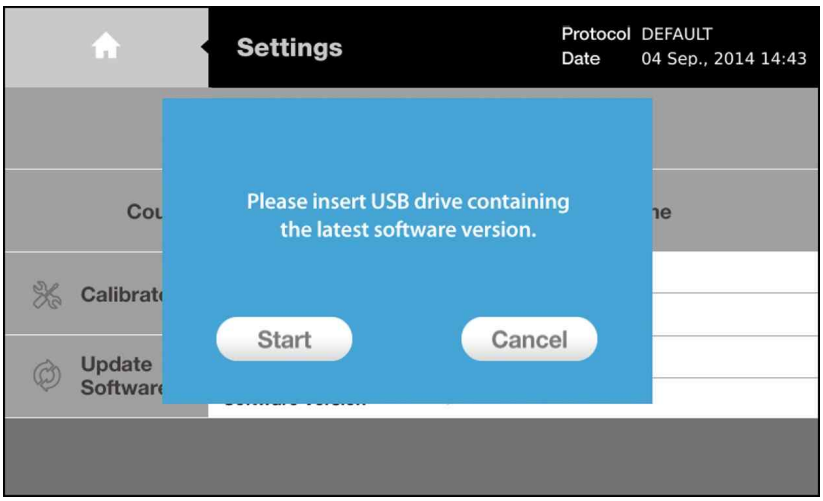
Logos Biosystems provides continuous update of software to maintain optimal performance.

The current version of software can be identified either in the Initializing screen (see page 15) or the Settings screen as above.

The up-to-date software, consisting of single file, can be downloaded from Logos Biosystems Website (http://www.logosbio.com). This software should be saved into the root directory of the USB drive. Before starting, download the new software into the Luna™ USB drive and connect the USB drive to the USB port of the instrument.

Press [Update Software] to start.

The following sign will be displayed on the touchscreen.



Press [Start]. Software update will be proceeded automatically. Do not turn off the instrument during update. After finishing, the current version of software and the date of last update will be changed automatically.

IMPORTANT

The re-calibration must be done after the software update. Please see Section 2.7.

Chapter 3 – Protocol Setting

The LUNA-II™ Automated Cell Counter provides a basic (DEFAULT) protocol which can be used for most common cell lines. In addition, LUNA-II™ also provides tools to setup individual protocols. All the parameters in the protocol can be modified by users and saved as alternative protocol. Users can save up to 300 protocols. These functions provide personalized protocol for optimal use.

3.1 Parameters in the Protocol

In the protocol menu, the LUNA-II™ Automated Cell Counter provides following parameters

Parameter	Range	DEFAULT
Dilution Factor	1 – 100	2
Noise Reduction	0 – 10	5
Roundness	0 – 100%	60
Min. Cell Size	3 – 59 μm	3
Max. Cell Size	4 – 60 μm	60
Declustering Level	None, Low, Medium, High	Medium

Dilution Factor: The value for dilution factor in the DEFAULT protocol is preset as 2 for With Trypan Blue. However, users can modify this value according to the dilution of the original sample. The Dilution Factor can be adjusted either by a scale of 1 or a scale of 10 between 2 to 10 and 10-100, respectively. The Dilution Factor is used to automatically calculate the concentration of cells in original sample from the cell counting result. Adjusting Dilution Factor will be helpful for users handling high density cells such as fermented CHO cells. In such cases, serial dilutions and repeated counting will be needed with appropriate Dilution Factor.

Noise Reduction: Noise Reduction means the decrease of the background for counting. With higher Noise Reduction, the instrument dose not detect faint signals of weakly stained objects. With lower Noise Reduction, the instrument can detect objects with faint signals. Since staining intensity of cells with trypan blue may vary from cell to cell, adjusting Noise Reduction will be helpful for optimal detection of specific type of cells.

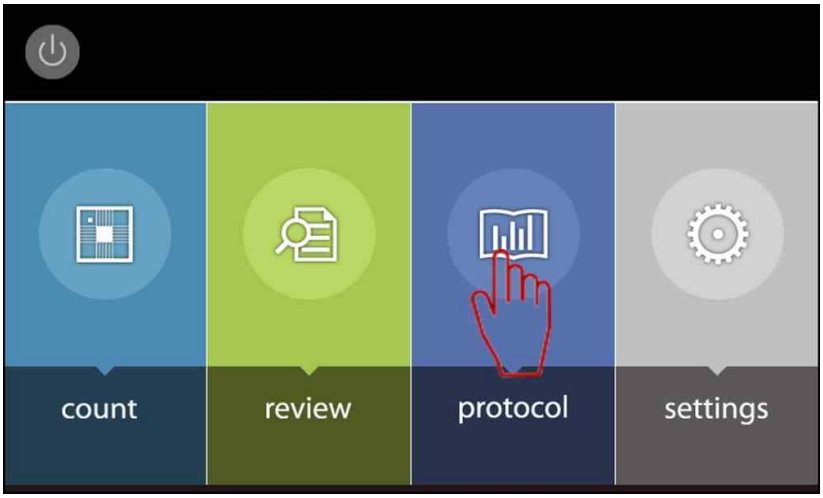
Roundness: Roundness refers to the roundness of the objects in the image. Since the shape of cells may vary and not complete sphere, adjusting Roundness enables optimal detection of cells. The instrument with higher Roundness counts objects with more roundness as cells and excludes objects with less roundness for counting as cells. LUNA-II™ with lower Roundness will be suitable to count cells with irregular shape since it detects objects with less roundness as cells.

Min. & Max. Cell Size: Average size of cells also may vary from cell type to cell type. With this parameter, users can optimize the instrument to efficiently detect their own cells. The value can be adjusted with 1 μm of increase per step.

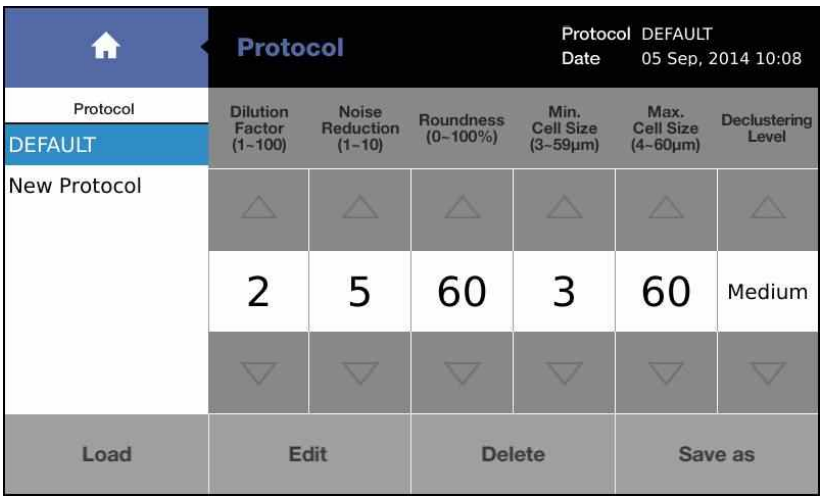
Declustering Level: Cultured mammalian cells may form clumps during culture or handling. Declustering function of LUNA-II™ provides efficient detection of clumped cells. LUNA-II™ provides 4 alternates: None, Low, Medium and High. This function is helpful to count sticky cells or rod-shaped spores. High level declustering takes more time to analyze.

3.2 Setting the Protocols

In the Start-Up screen, press [protocol] as below.



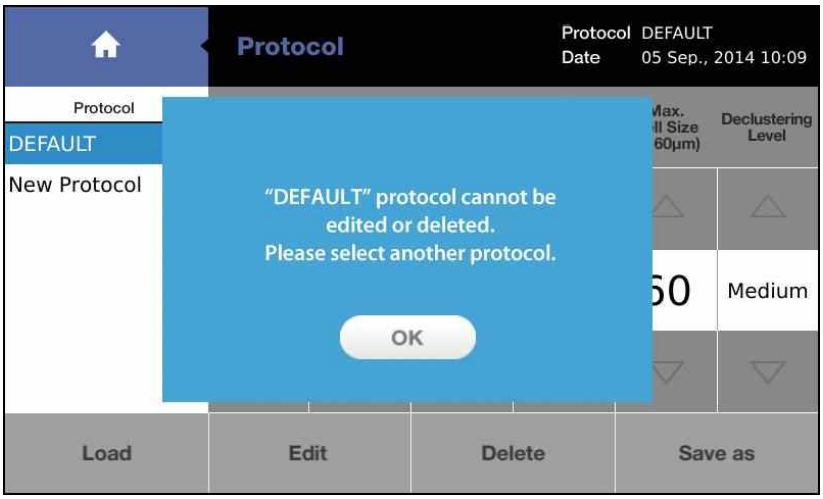
In the Protocol screen, users can identify all the parameters as below.



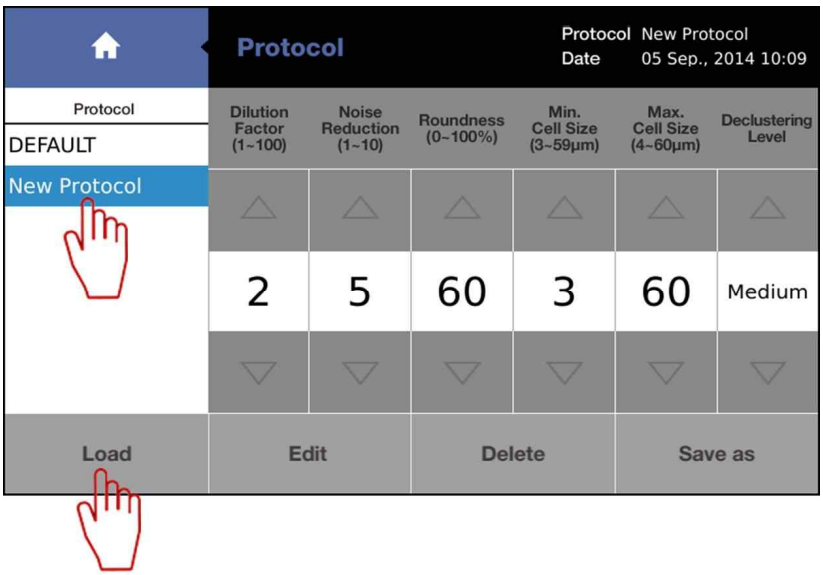
The selected protocol is marked with white letters and blue back ground in the left panel of screen.

The value of each parameter is displayed in the right panel of screen.

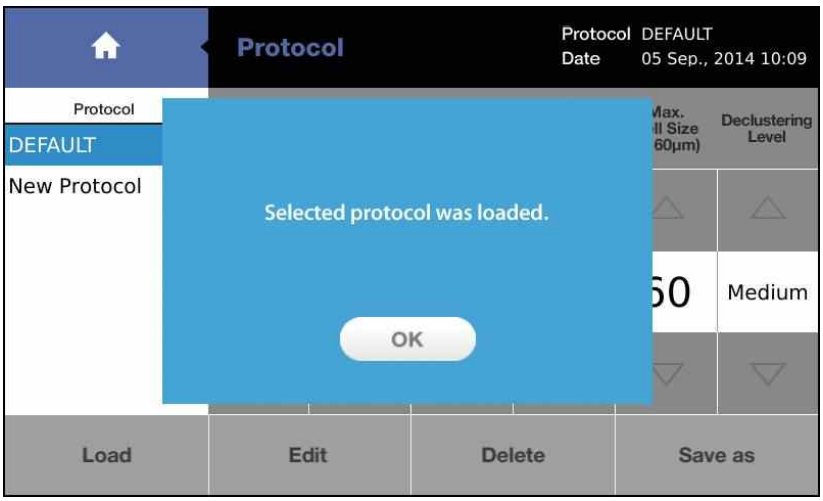
Note: The factory setting DEFAULT protocol cannot be modified.



Press other protocol or [New Protocol] and press [Load] as below.



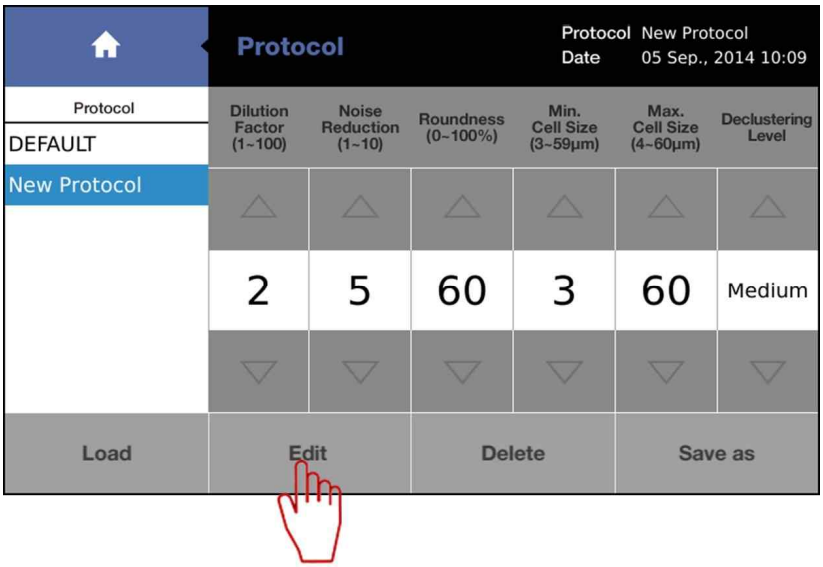
Now LUNA-II™ display following sign on the screen.



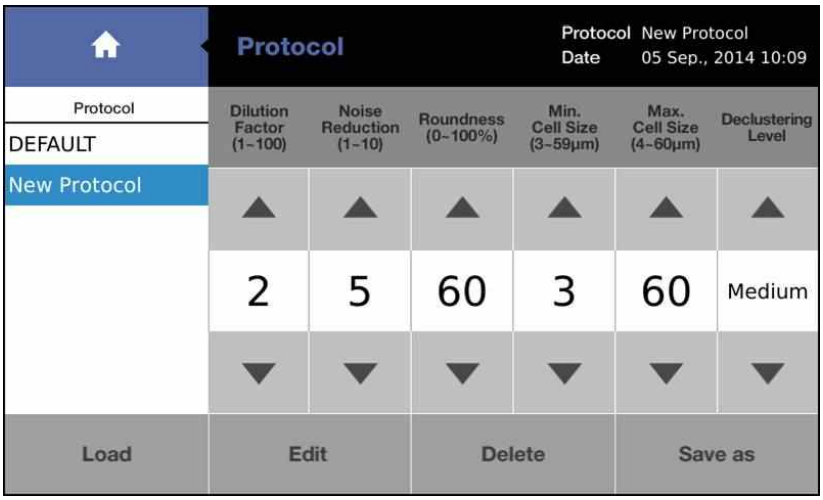
Press [OK].

To delete the selected protocol, press [Delete].

To edit the selected protocol, press [Edit].



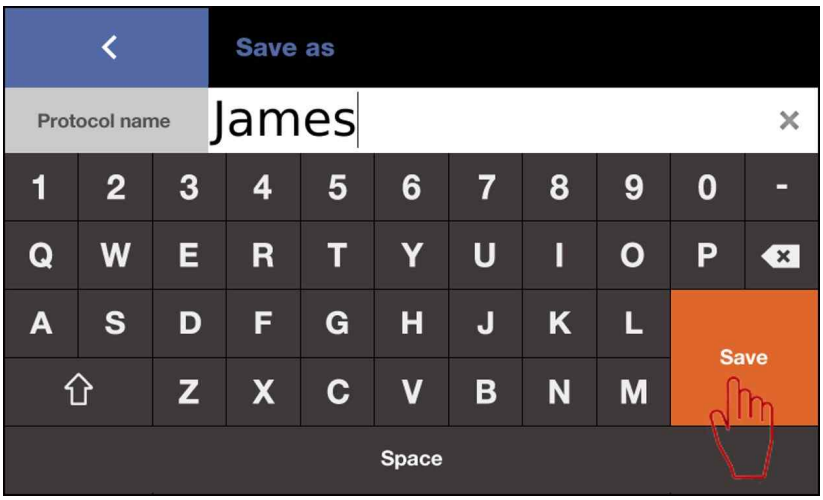
After pressing [Edit], the arrows (upward and downward) will be activated and marked as solid arrows as below.



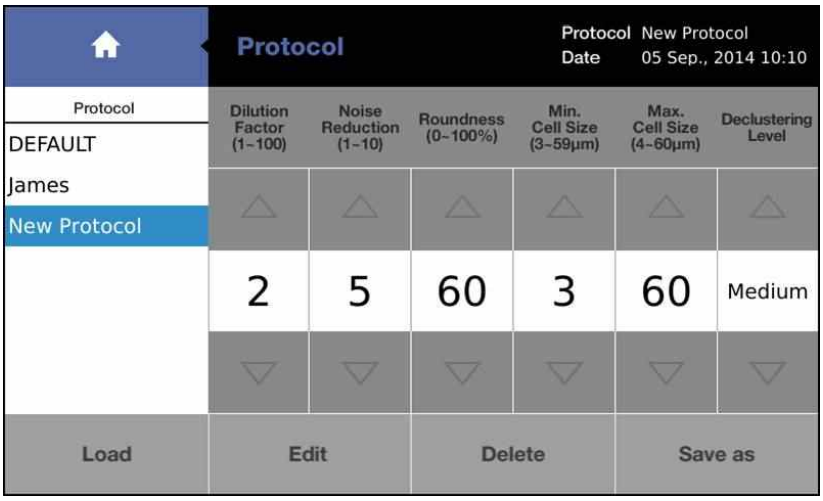
Press the arrows to edit the values. Then press [Save as].

Now users can see the Save as screen as below.

Using keyboard in the screen, put the desired name and press [Save] in the lower right corner.

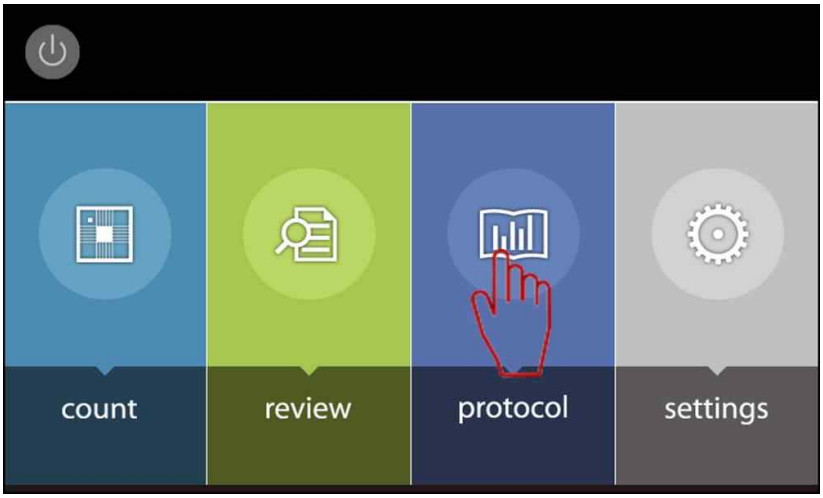


Now, new protocol named James is displayed in the Protocol panel of Protocol screen.

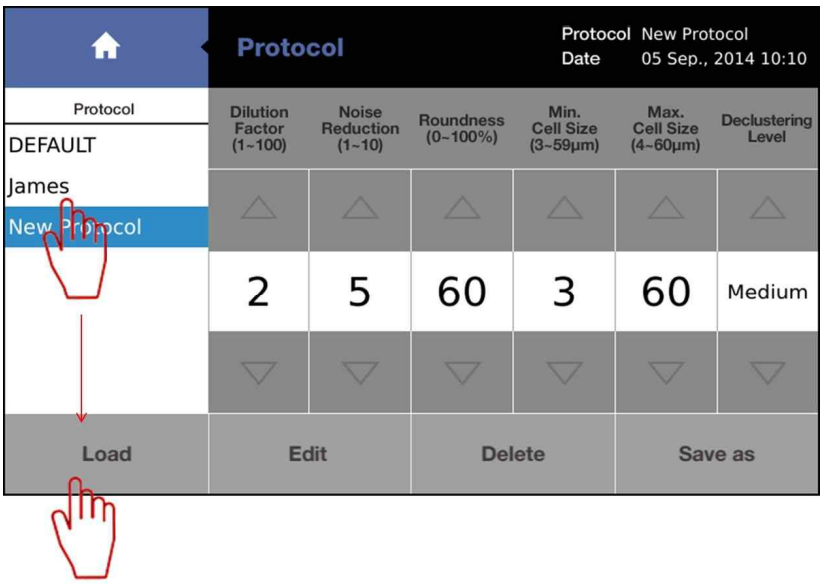


3.3 Selection of Protocol

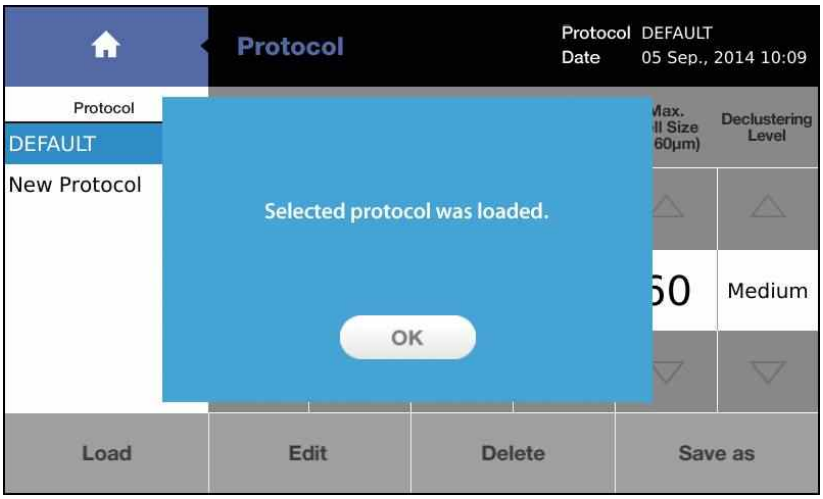
To change protocol to be used, press the protocol button in the Set-Up screen.



Select desired protocol by pressing the protocol name and press [Load] to apply selected protocol to use.



LUNA-II™ will display following sign on the screen.



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

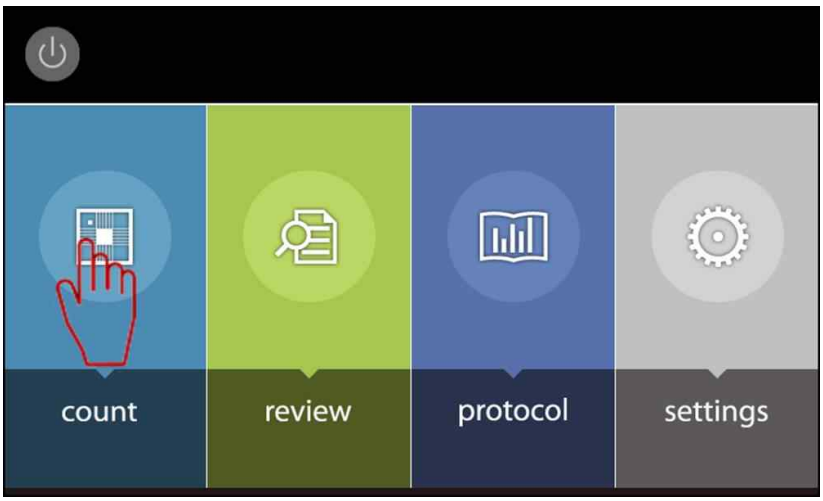
IMPORTANT

Selecting the protocol name does not mean changing protocol to be used. To use selected protocol, users should press [Load].

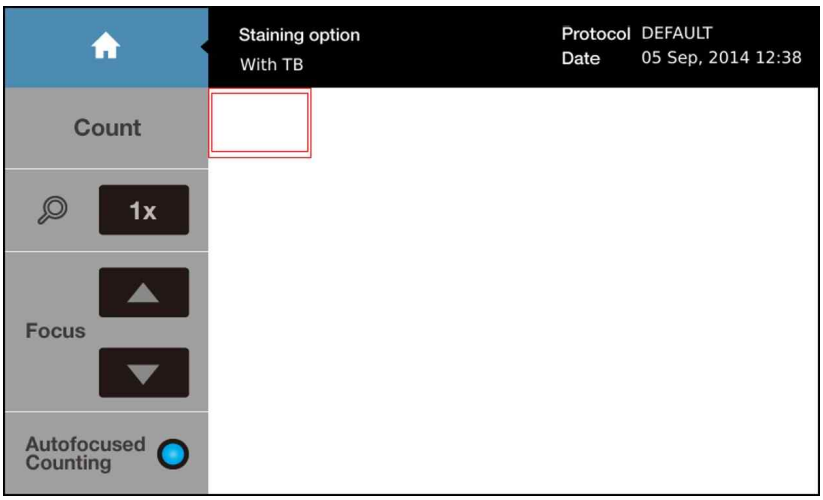
Chapter 4 – Counting Cells

4.1 Preparing the Instrument

Turn on or activate the LUNA-II™ Automated Cell Counter (See Section 7.1).
Press [count] on the Start-Up screen.



LUNA-II™ will display the Count screen.



In the Count screen, users can identify the Staining option and selected protocol in the upper panel of screen. To change the Staining option, see Section 2.5 Changing Options for Cell Counting. To change the Protocol, see Section 3.3 Selection of Protocol.

Users can also identify the date and time in the upper right corner of screen. To change the date and time, see Section 2.6 Setting the Date and Time.
Now LUNA-II™ is ready to count.

4.2 Sample Preparation

4.2.1 Materials required

- Cell suspension
- Luna™ Cell Counting Slides
- Trypan Blue Stain 0.4 %
- Luna™ USB drive or equivalent

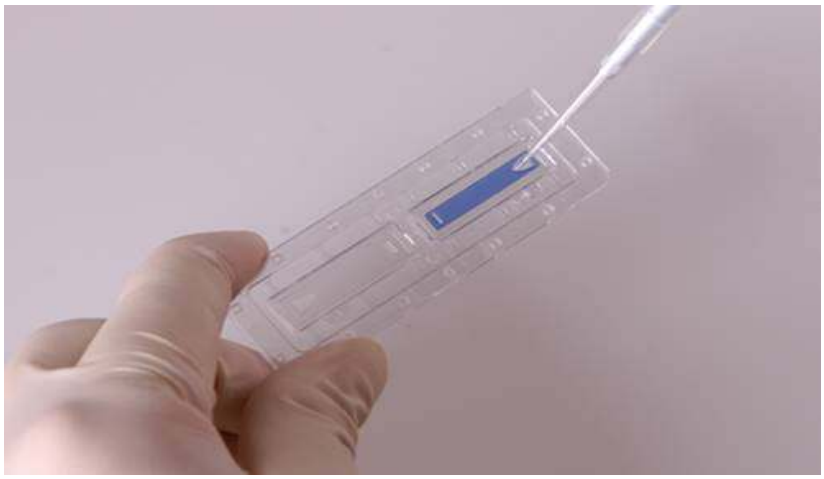
4.2.2 Prepare cell suspension according to standard cell biology procedure. Please avoid clumping of cells for accurate counting.

4.2.3 Mix 10 μl of the cell suspension with 10 μl of Trypan Blue Stain in an appropriate tube by gentle pipetting up and down.

4.3 Loading Samples into the Luna™ Cell Counting Slide

Hold the edge of the slide and load 10 – 12 μl of the mixed cell sample into the sample loading port of one chamber of the counting slide. Alternatively, the slide can be placed on a clean surface during sample loading.

For easy and accurate loading, tilt the pipette 45 – 60 degrees as shown below.



Note: Be careful not to over-load or under-load the sample into the chamber.

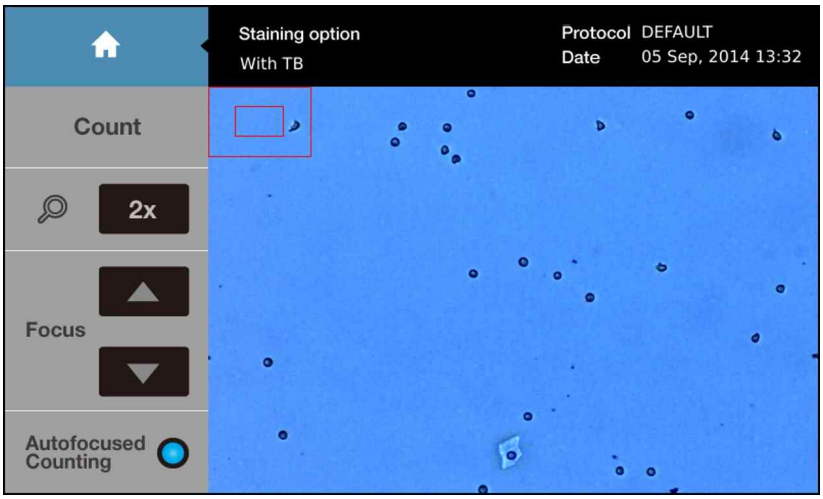
4.4 Counting Cells

4.4.1 Insert the sample-loaded slide into the counting slide port of LUNA-II™, ensuring that the loaded chamber is inserted into the instrument. LUNA-II™ analyzes only the inserted chamber upon counting.

Note: After inserting the slide, LUNA-II™ only reads the inserted chamber.

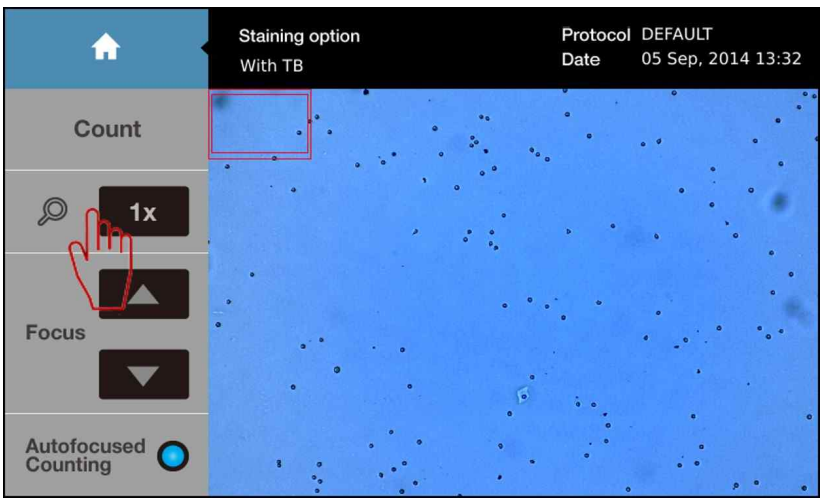
Note: Make sure that the counting slide is not inserted upside-down.

Now users can see the cells on the screen as below.

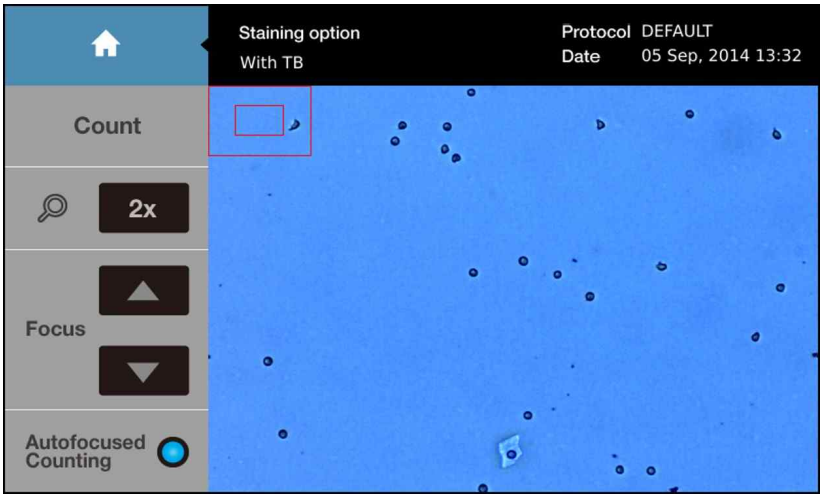


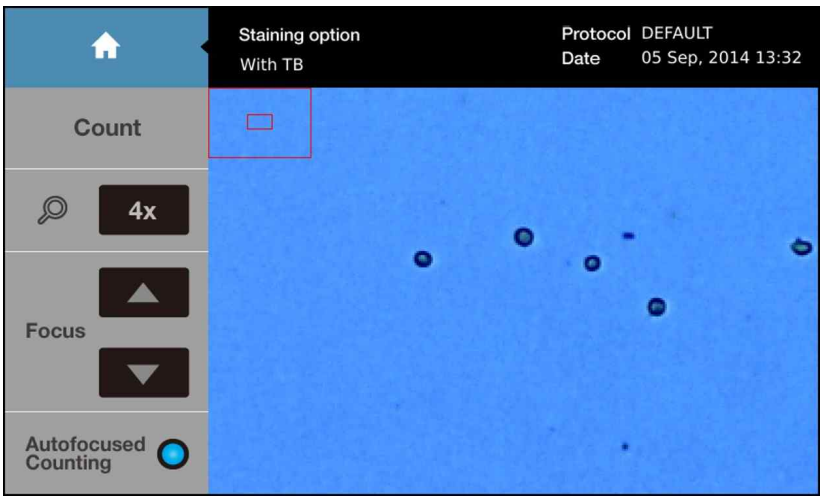
Note: If users cannot see the cells, the cell counting slide may not be inserted correctly.

The magnification of cells can be changed by pressing the magnifier as below.



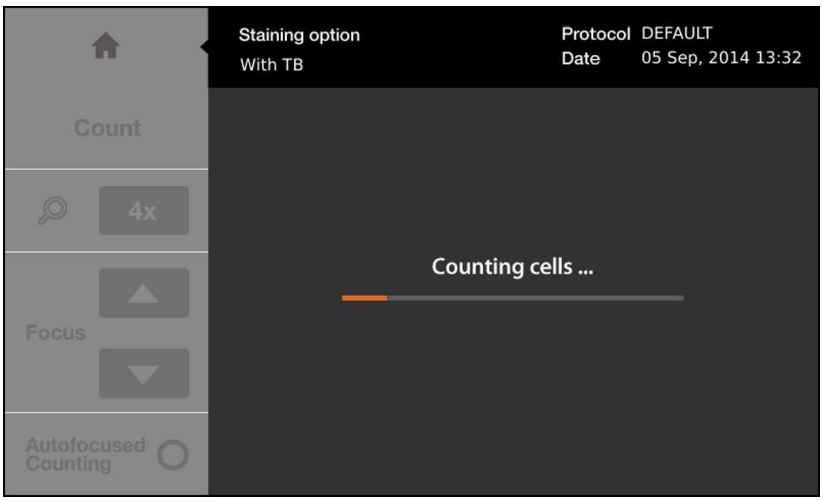
The LUNA-II™ Automated Cell Counter provides 1X, 2X, and 4X images as follows.



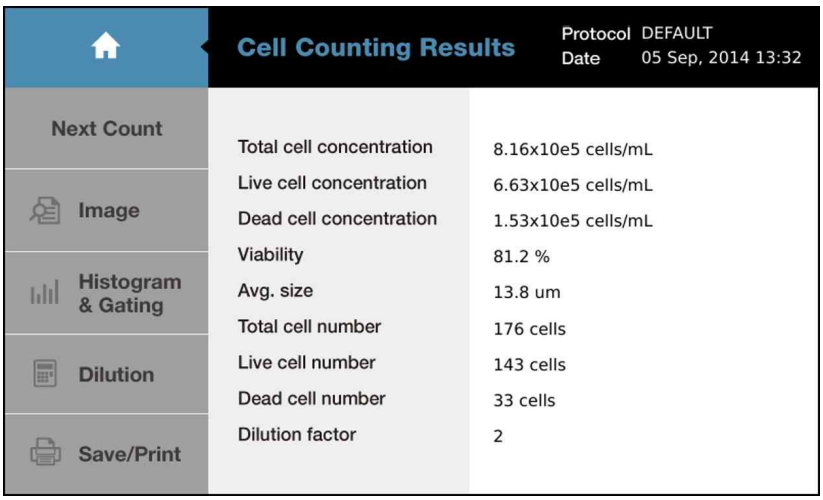


The field of cell image can be navigated by pressing the screen and moving the finger or stylus pen on the image. Two small red boxes in the upper left corner of cell image represent the entire field and field of view, respectively. The outer box represents the entire field of image. And the inner box represents current field of view. Upon navigating the image, the location of inner box will be changed.

4.4.2 Press [Count] to start counting. Now all of the buttons will be inactivated and the bar, which is indicating the progress of cell counting, will be appeared on the screen as below.

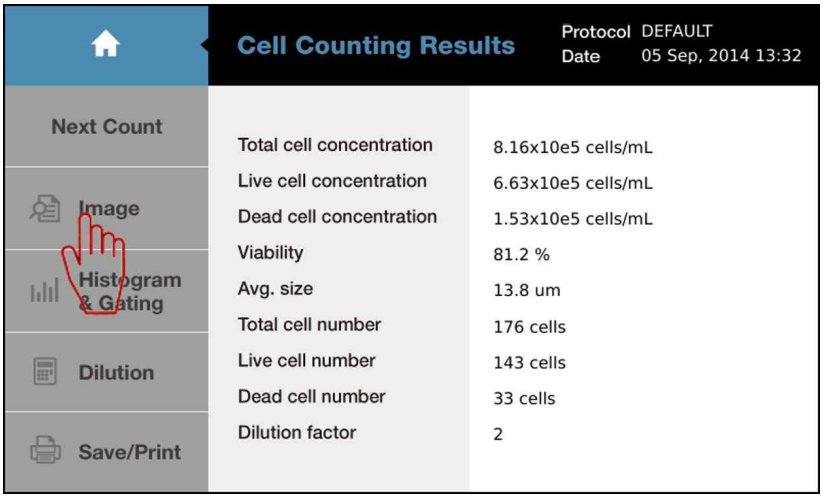


In general, the counting takes 15 to 22 seconds. LUNA-II™ will display the Cell Counting Results screen as below,

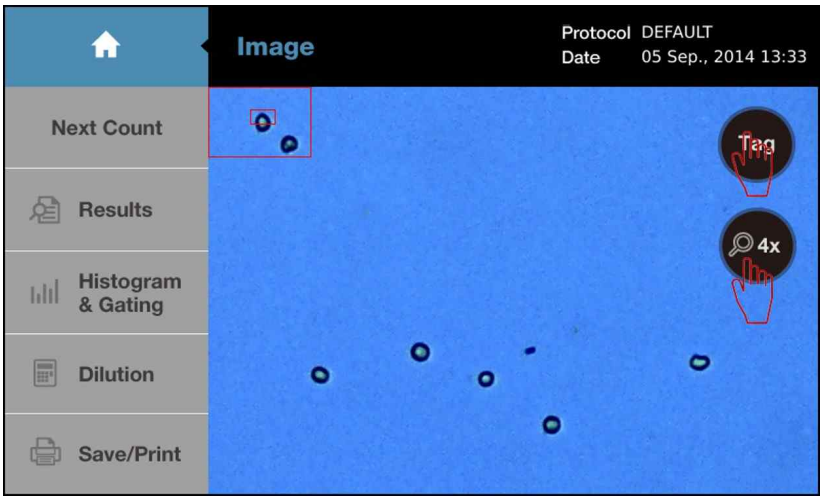


4.5 After Counting: Image View

To view the captured image of cells, press [Image] as below.



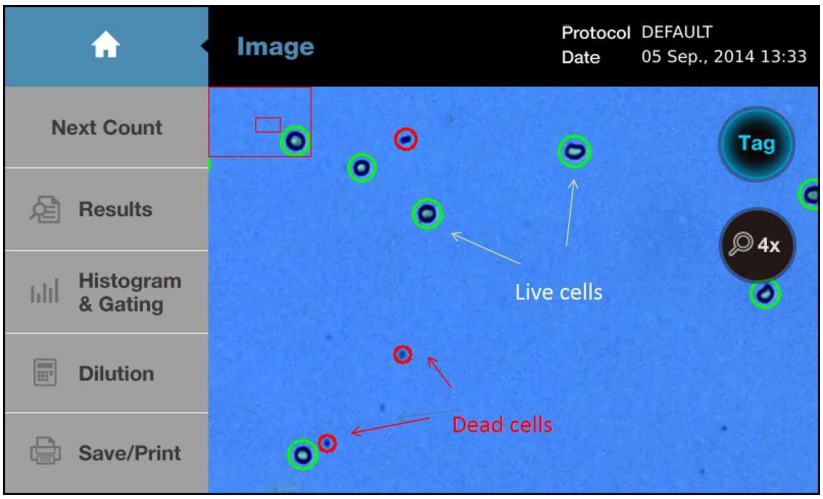
The Image screen will be appeared as below.



Similar to the Count screen, the magnification of the captured image can be changed by pressing the magnifier button on the right of the image. The LUNA-II™ Automated Cell Counter provides 1X, 2X, and 4X magnification of the image.

The captured image is also able to be navigated by pressing the screen and moving the finger or stylus pen on the image. Two small red boxes in the upper left corner of cell image represent the entire field and field of view, respectively. The outer box represents the entire field of image. And the inner box represents current field of view. Upon navigating the image, the location of inner box will be changed.

[Tag] in the upper right corner of the image will activate the Tag function. The live cells will be marked by green circles and dead cells will be marked by red circles as below.

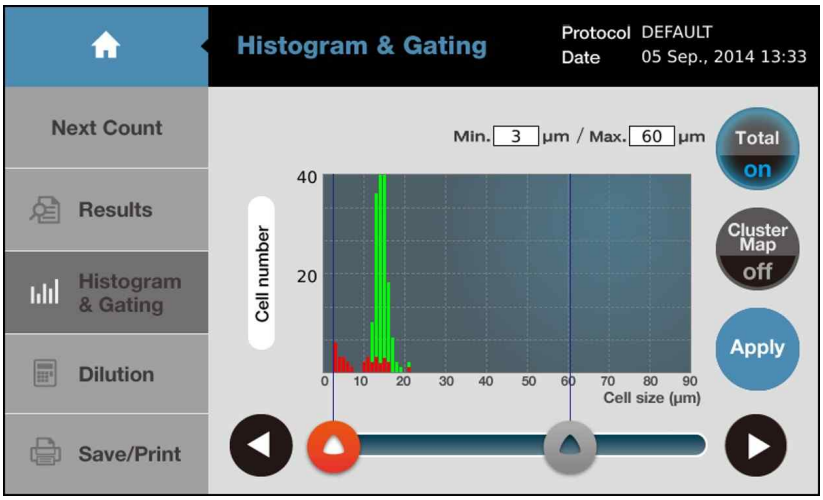


Note: This “Tag” function is one of the distinct tools of the LUNA-II™ Automated Cell Counter. This function allows the user to review the data immediately to determine the accuracy of the counting without additional manipulation or devices.

After reviewing the image of cells, “Tag” button can be pressed again to remove the green and red circles from the image.

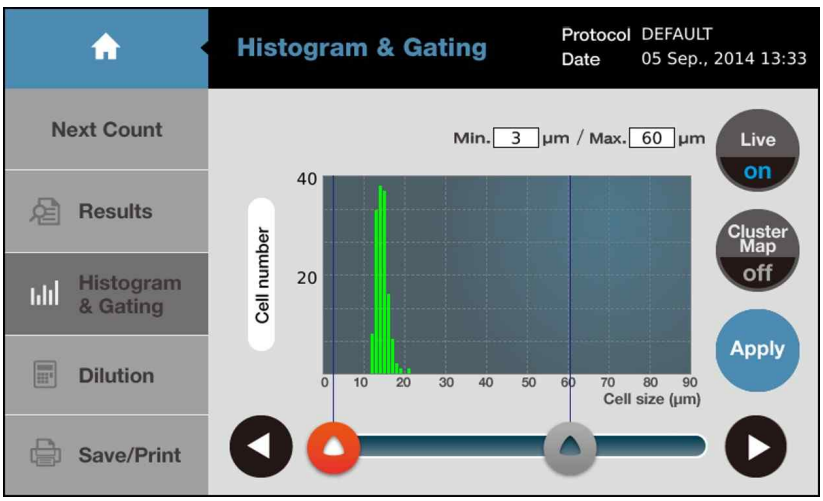
4.6 After Counting: Histogram & Gating

LUNA-II™ provides graphical analyses of the cell counting results. By pressing Histogram & Gating, you can view the Histogram & Gating screen as below.

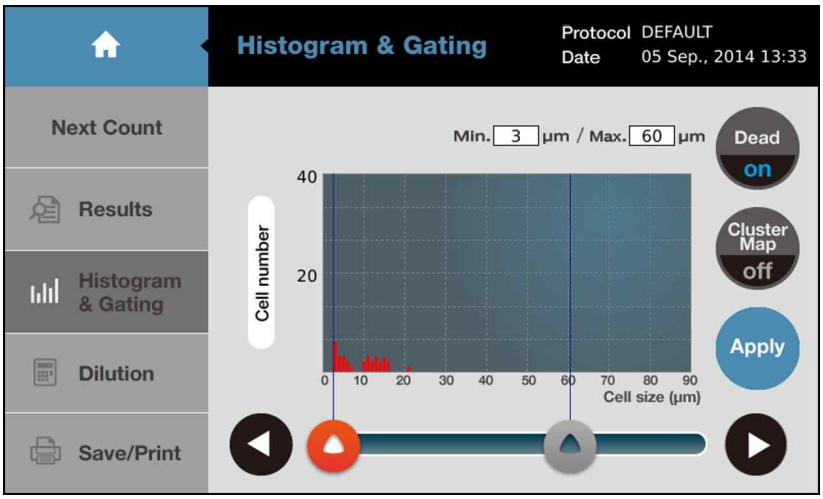


On this screen, users can review the distribution of cells according to their sizes. Green bars represent the live cells and red bars represent dead cells.

The histogram will change by pressing two buttons on the right side of screen. When pressing [Total/on], the icon will be changes as [Live/on] and the histogram will display the distribution of live cells without dead cells as below.

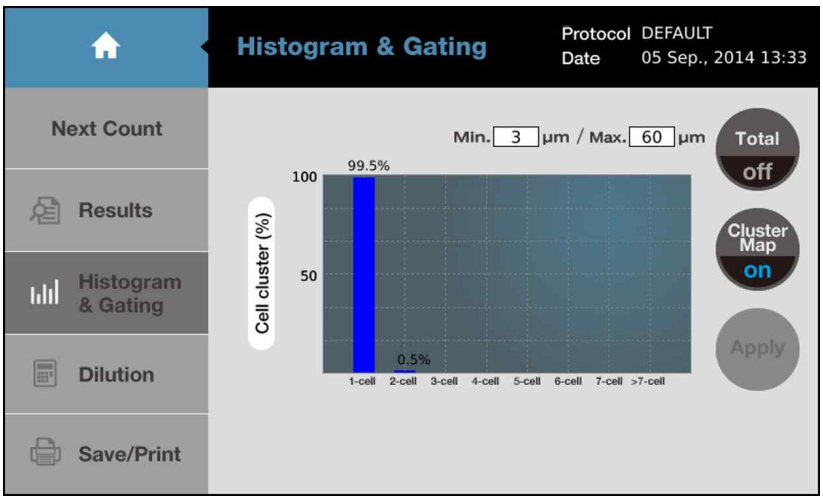


When pressing [Live/on], the icon will be changes as [Dead/on] and the histogram will display the distribution of dead cells without live cells as below.

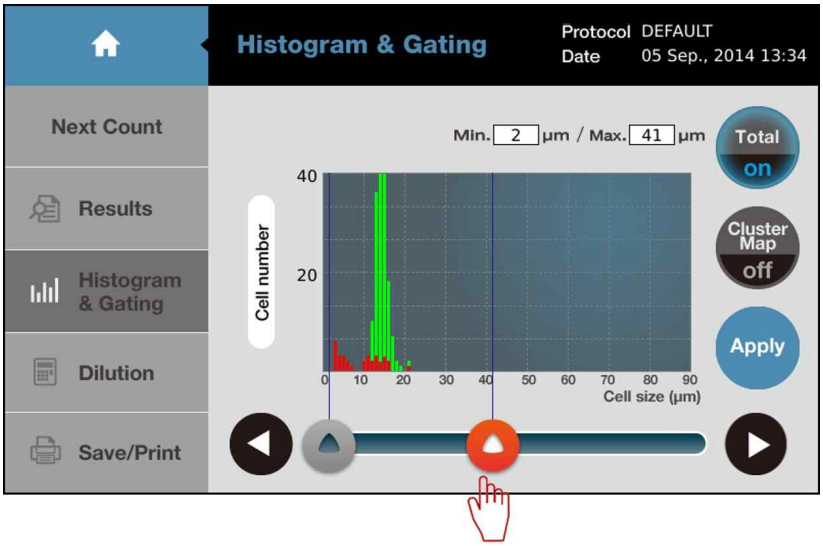


In addition, when pressing [Cell number] on the left side of histogram, the LUNA-II™ Automated Cell Counter also display the cell concentration.

The LUNA-II™ Automated Cell Counter also provides the distribution of clusters as below after pressing [Cluster Map/off].



In addition, the LUNA-II™ Automated Cell Counter provides gating function. Gating function consists of four components: the lower and upper limit and two arrow head in the lower part of screen as below.



The lower and upper limit can be activated by pressing as above and adjusted by pressing the icons with arrow head.

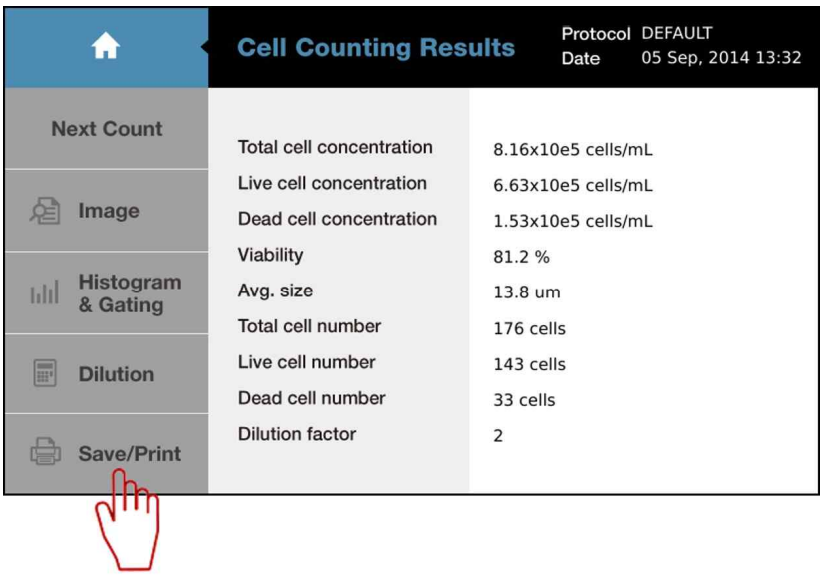
The gating function will be helpful to analyze the cells from co-culture of cells with distinct sizes of cells.

The gating function will be also helpful to exclude non-cellular particles with distinct sizes from various tissue engineering applications.

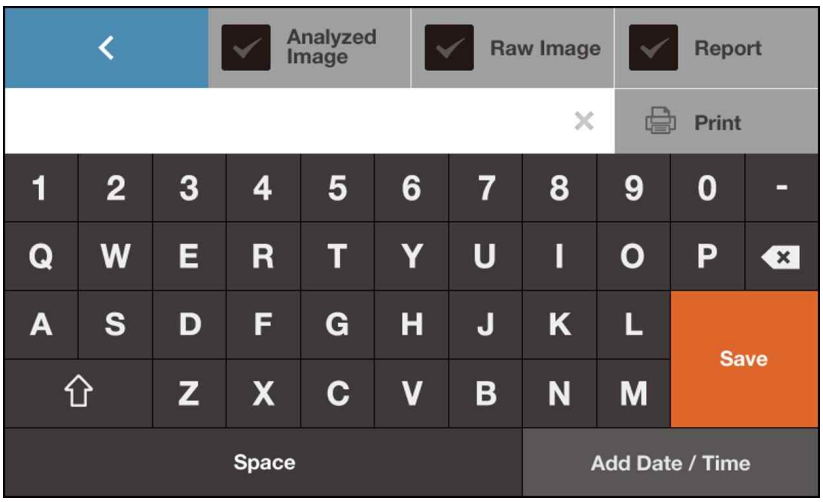
4.7 After Counting: Saving and Printing the Results

The LUNA-II™ Automated Cell Counter provides multiple record options: saving the results and/or printing the results.

To save or print the results, press [Save/Print] in the Cell Counting Results screen as below.

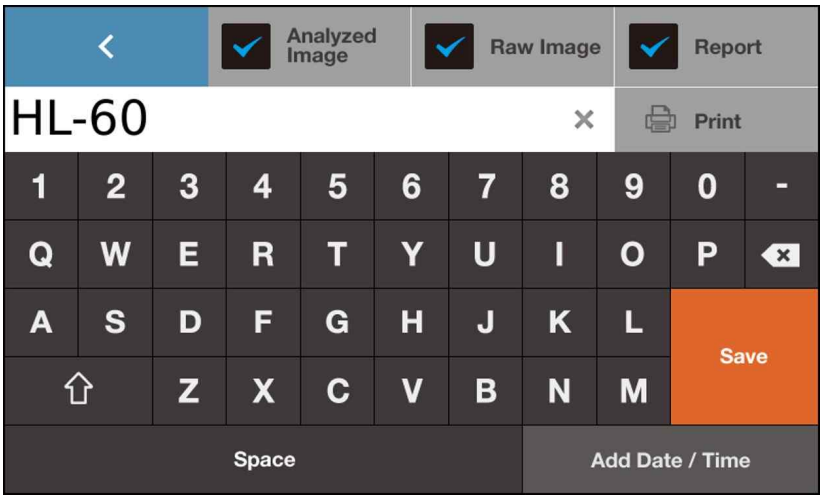


The Save/Print screen will be seen as below. In the upper part of screen, three items will be displayed in inactive status.



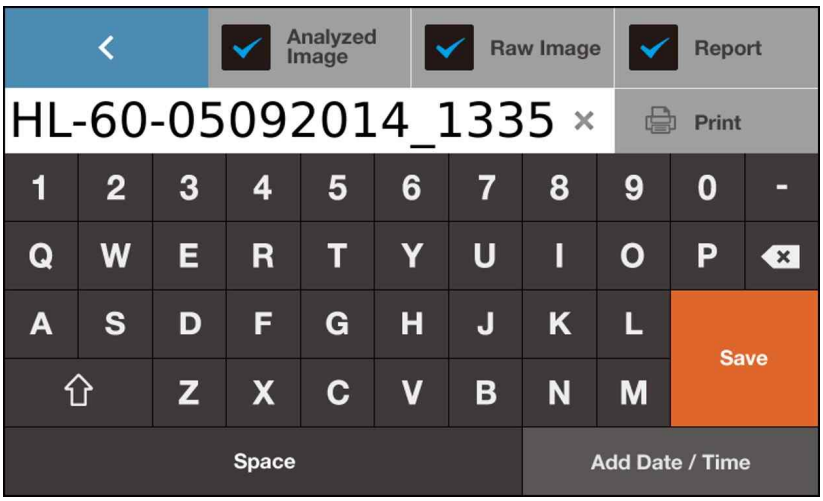
Items	Description
Analyzed image	The image of cells with tags of live and dead cells.
Raw image	The image of cells without tag.
Report	The PDF report containing counting results and histograms

These items can be activated by pressing and displayed with blue √ marks as below.



Put appropriate name such as the name of cells using keyboard on the screen as above.

Optionally, users can put the date and time by simply clicking [Add Date / Time] in the lower right corner of screen.



Now, users can save the selected data by pressing [Save] in the lower right corner of screen.

IMPORTANT

Remember that the “Raw Image” should be turned on to save the “Raw Image” which should be provided to your distributor or Logos Biosystems to get the best technical support.

The LUNA-II™ Automated Cell Counter provides additional option to report the results. Users easily print the Cell Counting Results by simply pressing [Print] in the upper right corner of screen. The printed report will be displayed as below.

Cell Count Report

File name: HL-60-05092014-1

3350605

Date: 05 Sep., 2014 13:35

Cell count results

[Total]: 8.16x10e5 cells/mL

[Live]: 6.63x10e5 cells/mL

[Dead]: 1.53x10e5 cells/mL

Viability: 81.2 %

Avg. size: 13.8 um

Total #: 176 cells

Live #: 143 cells

Dead #: 33 cells

Dil. Factor: 2

Protocol

Protocol name: DEFAULT

Noise reduction: 5

Roundness: 60

Min. cell size: 3

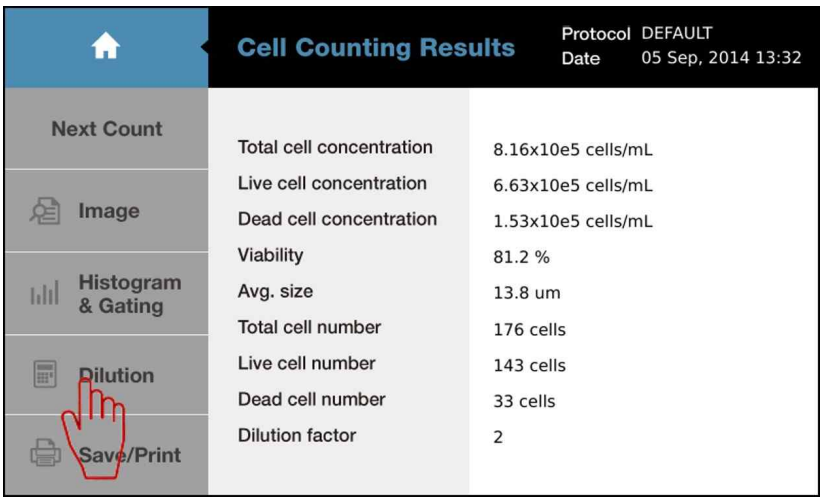
Max. cell size: 60

Size gating: 3 ~ 60 um

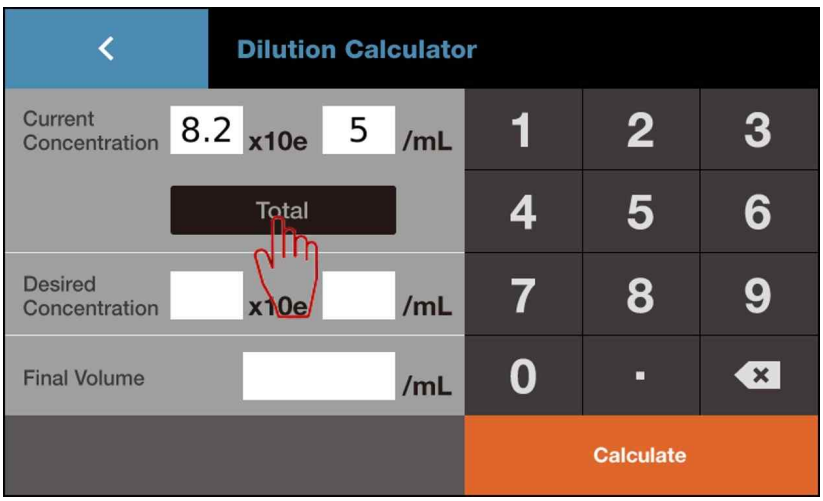
4.8 After Counting: Calculation for Subsequent Experiments

The LUNA-II™ Automated Cell Counter provides a built-in dilution calculator. Users easily calculate the amount of cell suspension to dilute for subsequent experiments.

Press [Dilution] to activate the Dilution Calculator as below.



The Dilution Calculator initially shows the measured concentration of total cells from cell counting. Users can select the Current Concentration of Total, Live, or Dead cells by pressing the Black Sign under the value as below. Put the appropriate numbers into the blanks of the “Desired Concentration” and “Final Volume” that you want to obtain. Then click [Calculate] in the lower right corner of screen.



Chapter 5 – Focusing Option

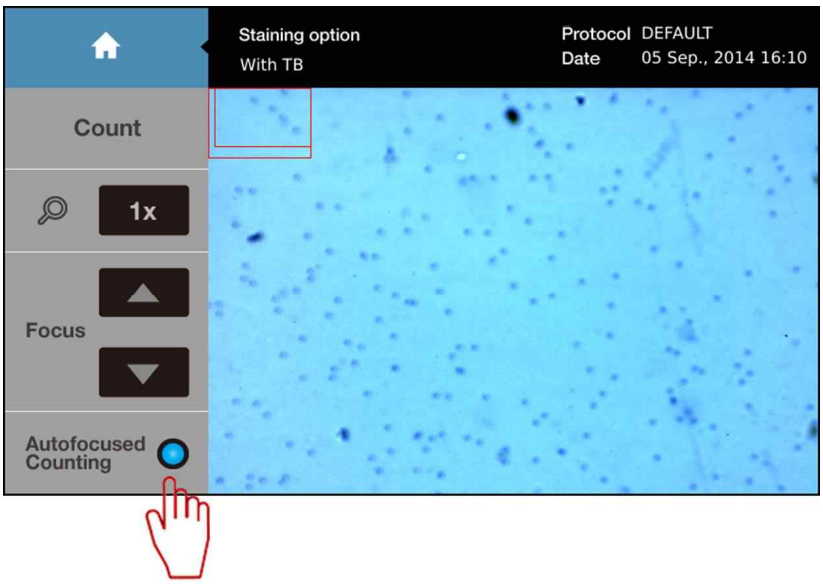
The LUNA-II™ Automated Cell Counter has been integrated with a novel focusing mechanism based on the liquid lens technology. Unlike traditional autofocusing based on mechanical moving of lens in vertical axis, the liquid lens technology enables autofocusing without mechanical moving.

With this advance, the LUNA-II™ Automated Cell Counter provides two focusing options: Autofocusing and manual focusing.

5.1 Autofocusing

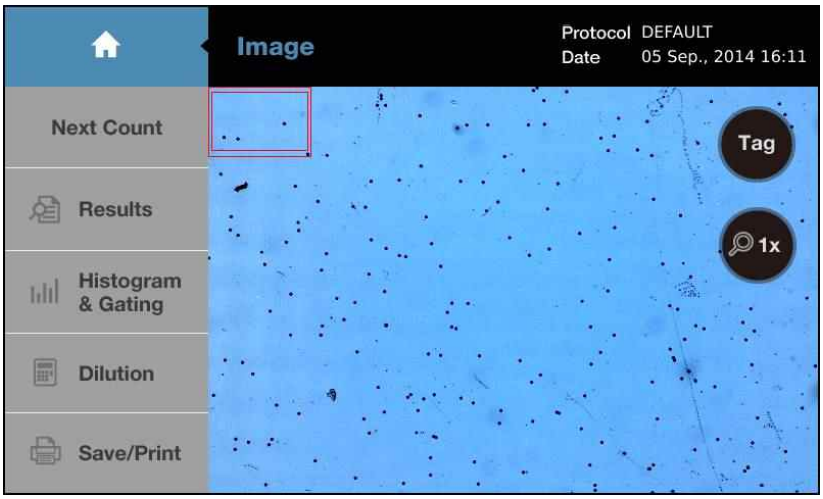
With autofocusing function, the LUNA-II™ Automated Cell Counter achieves true automation of cell counting. Users count cells by simply insert the cell counting slide and pressing [Count] without tedious focusing process. In addition, autofocusing function eliminates potential human error.

Autofocusing function can be activated by simply pressing [Autofocused Counting] in the lower left corner of the Count screen. When activated, the blue circle will be displayed on the right side of [Autofocused Counting] as below.



As shown above, the Luna™ Standard Beads are out-of-focus. However, users can count the beads without tedious focusing.

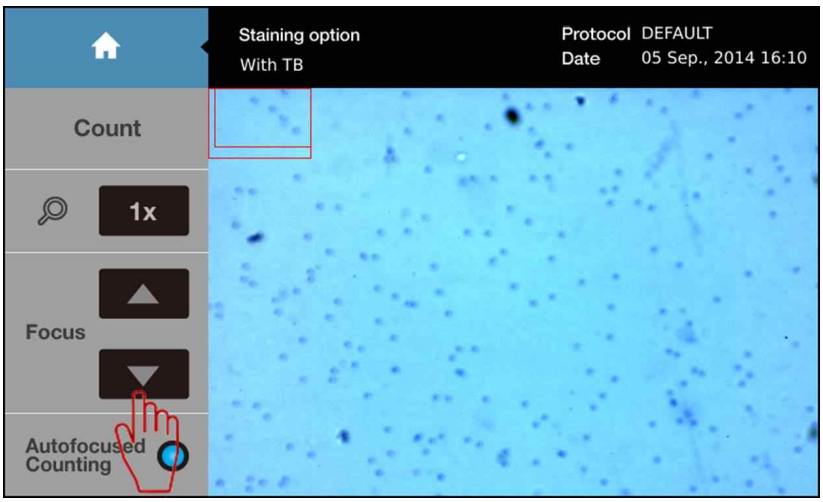
After pressing [Count], users can obtain autofocused image as below.



5.2 Manual Focusing

Although the LUNA-II™ Automated Cell Counter provides autofocusing function, users may want to focus the image manually with various purposes.

The LUNA-II™ Automated Cell Counter also provides manual focusing function. Users can adjust the focus manually by simply clicking the arrow heads (up or down) as below with either activating or deactivating autofocus function.



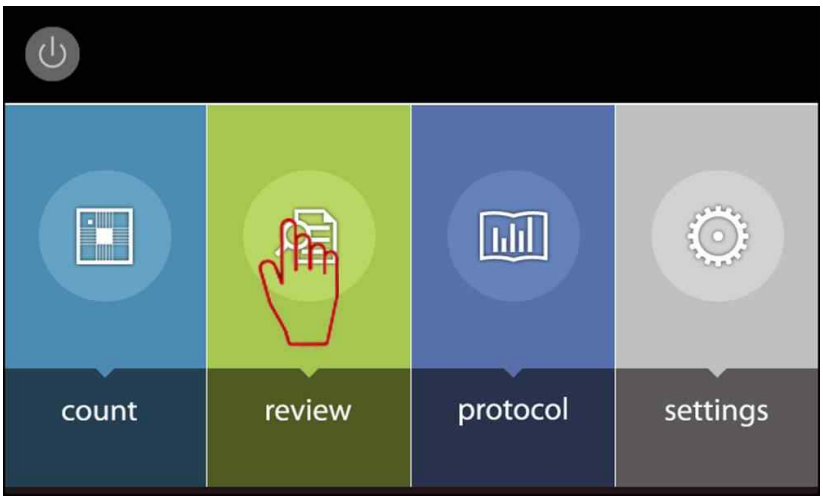
Note: If needed, users can count the cells with deactivating autofocus function after manual focusing.

Chapter 6 – Review the Previous Results

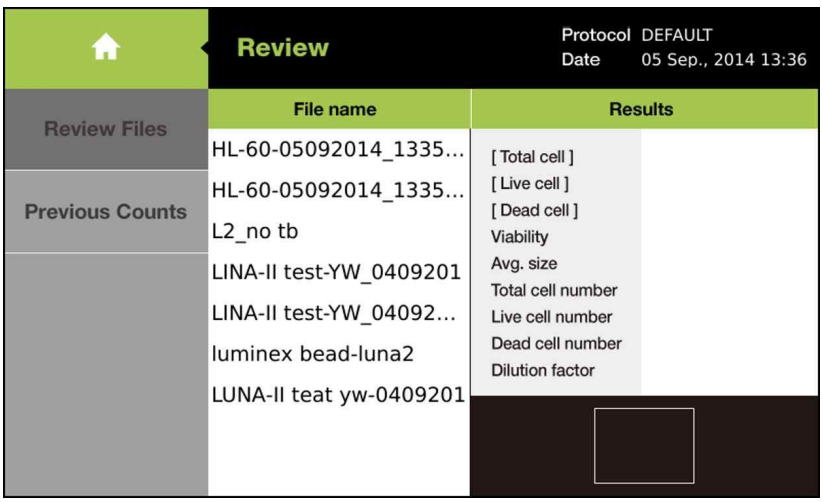
The LUNA-II™ Automated Cell Counter provides stand-alone review function for previous results. Users can easily review the previous results without additional devices.

To review the previous results, the USB drive that contains the previous results should be connected to the USB port of the LUNA-II™ Automated Cell Counter.

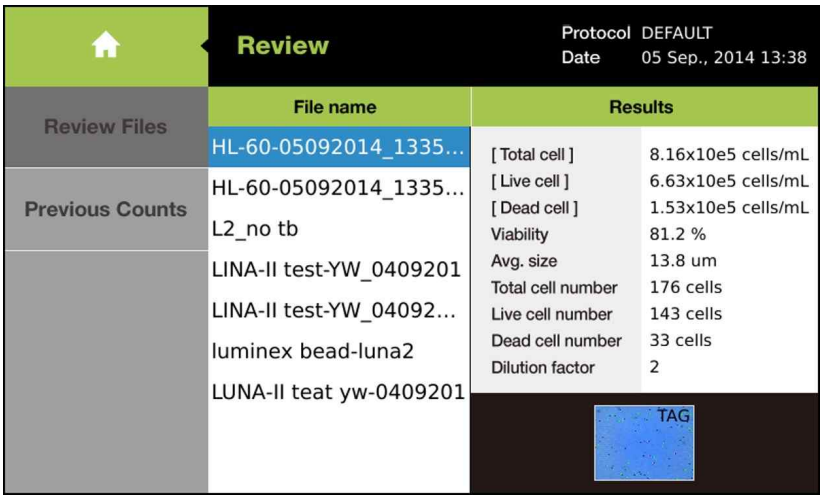
Users can select the Review function by clicking [review] in the Start-Up screen.



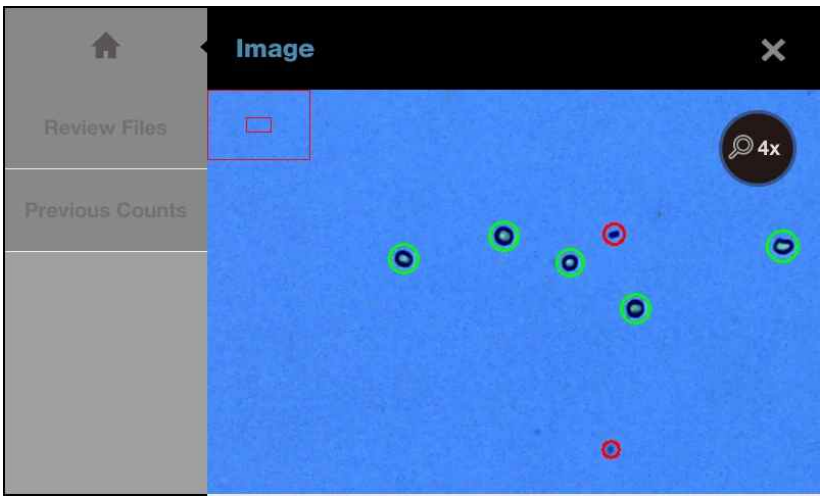
The Review function provides two options: Review Files and Previous Counts as below.



In the Review Files, users can select File name to review the results and image. After selecting the file name, the previous cell counting results will be displayed on the right side of screen as below.

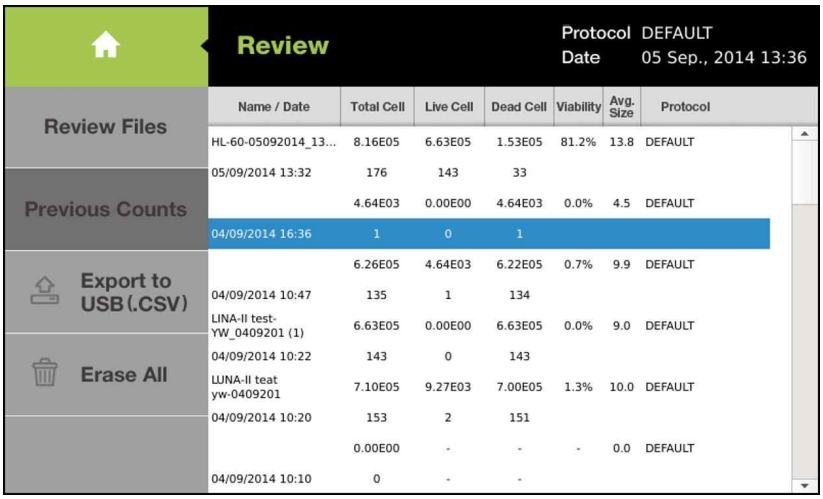


The captured image with tags can be also reviewed by pressing the small image under the Results section as above.



The magnification of image can be adjusted by pressing the magnifier on the image.

In the Previous Counts function, users can overview the list of previous counts with summarized results as below. This list can be exported into USB drive as a CSV format.



The LUNA-II™ Automated Cell Counter can store up to 1,000 previous counts.

Chapter 7 – Maintenance and Troubleshooting

7.1. Turn On/Off LUNA-II™

To turn on LUNA-II™, press the POWER button in front of the instrument.

The Power icon in the Start-Up screen can be used to turn off the instrument (see page 17).

Alternatively, the instrument is turned off by pressing the POWER button in front of the instrument for 5 seconds.

Since LUNA-II™ provides standby mode, it is not necessary to turn off the instrument in general laboratory use. Standby mode will be activated after 10 min of inactivity.

In the standby mode, the touchscreen will blackout. By simply pressing the touchscreen or the POWER button, the LUNA-II™ will be ready to use with displaying the last screen.

7.2 Cleaning

Generally, the LUNA-II™ Automated Cell Counter does not require regular maintenance for appropriate operation. However, if the instrument is used for long periods of time and continuously, it may need to be cleaned or decontaminated to remove any dirt or dust on the surface of the instrument. Be sure to turn off the LUNA-II™ Automated Cell Counter and disconnect the power cable before cleaning or performing any other maintenance. Ensure that water and other solutions do not enter any part of the instrument during cleaning.

7.2.1 Cleaning the surface

With a soft and damp cloth, wipe the surface of the instrument. Use some distilled water or alcohol for dampening the cloth. After cleaning, immediately dry the cell counter with a dry cloth. Do not wet the instrument by pouring or spraying water or other liquids directly on the instrument. In particular, power-related parts should never become wet in order to avoid electrical shock or damage.

7.2.2 Cleaning the touchscreen

Gently wipe off the touchscreen with a soft cloth lightly moistened with an authorized LCD cleansing detergent. Since excessive force or pressure on the touchscreen can cause damage, be gentle and cautious during cleaning. Wipe the touch screen dry immediately.

7.2.3 Decontaminating with alcohol

When the instrument needs to be decontaminated, use a soft cloth lightly moistened with 70% alcohol to wipe the outer surface. Never pour or spray alcohol or any other solution directly onto the instrument; this may cause severe damage to the instrument or give an electric shock to users.

Note: do not use an abrasive solution or a bleach solution that can cause scratches on the outer surface or the touchscreen.

7.3 Calibrating the Touchscreen (see section 2.4)

7.4 Calibrating LUNA-II™ (see section 2.7)

7.5 Updating the Software (see section 2.8)

IMPORTANT

The re-calibration must be done after the firmware update. Please see Section 2.3.4.

7.6 Troubleshooting

Problem	Possible Cause	Solution
Inaccurate cell count	Clumped cells	Make sure that cells are not clumped. The more single cells, the better counting results.
	Concentration of cells	For best results, the concentration of cells should be 5 x 10^4 – 1 x 10^7 cells/ml. If needed, dilute or concentrate the cells within this range.
	Insertion of counting slide	Ensure that the counting slide is inserted properly into the instrument.
	Sample loading	If the counting slide is over- or under-loaded with the sample, it may affect counting results. The optimal volume of sample is 10 – 12 μl of cell suspension.
	Malfunction of optical components	Any of the optical components may be damaged. Or, the objective lens may be dirty due to dust, spilled samples, or unknown causes. Please contact your local supplier.
	Damage or contamination of counting slide	Make sure that the counting area of the slide is transparent before loading the sample. Wear gloves while handling the slide.
Data transfer and saving	Incompatible USB drive	Use the USB drive supplied with the instrument. Or, make sure that your USB drive is compatible with the instrument. The version of the USB drive must be 2.0. Some types of USB drives are not detected or compatible with the instrument.
	Too many files in the USB drive	When there are too many saved files on the USB drive, reading and writing by the counter may slow down.
Errors during updating and calibrating the instrument	Freezing during calibration	Generally, re-calibration takes several minutes. However, it may take more time, depending on the extent of background adjustment. If the calibration takes more than 10 minutes, reset the system by turning off and on using the power button located in front of the instrument. Please contact service engineer if the calibration fails repeatedly.

	Incompatible USB drive	Use the USB drive supplied with the instrument. Or, make sure that your USB drive is compatible with the instrument. The version of the USB drive must be 2.0. Some types of USB drives are not detected or compatible with the instrument.
	More than one software version	Delete software with previous versions from the USB drive before downloading new software.
	Incorrectly saved or damaged software	First, make sure that the USB drive works well and is compatible with the instrument; Second, download the file again onto the USB drive. The file should be located in the root directory; Third, ensure that the USB drive is inserted correctly; Last, try the update again. If the problem continues, contact your local supplier.

Chapter 8 - Ordering Information

The following products can be ordered from your regional supplier or the website (www.logosbio.com).

Cat #	Product	Size
L40001	LUNA-II™ Automated Cell Counter (with printer)	each
L40002	LUNA-II™ Automated Cell Counter (without printer)	each
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% (for use with LUNA-II™)	2 x 1 ml
B13001	Luna™ Standard Bead	2 x 1 ml
U10004	Luna™ USB Drive (4 Gigabytes)	each
P12001	LUNA-II™ Printer Paper (10/pk) – min 700 prints	each

Chapter 9 - Purchaser Notification

9.1 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 re-sell 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 e-mail to info@logosbio.com for more information.

9.2 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.

Contact Information

For more information or technical support, please call, write, fax, or email. Our regional suppliers are listed on our web page (www.logosbio.com).

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