LUNA-FL™ Automated Fluorescence Cell Counter

User Manual

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IMPORTANT

LUNA-FL™ Automated Fluorescence Cell Counter is a Laboratory Electrical Instrument for Scientific Research Use Only. It is NOT A MEDICAL or IN VITRO DIAGNOTICS DEVICE.

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Safety Information

For the best results, users of the LUNA-FL™ Automated Fluorescence Cell Counter (or, hereinafter "LUNA-FL™") must follow the instructions below in addition to the general precautions for using electrical instruments.

1. Users must be careful to avoid electric shock while operating the instrument. Do not touch it or its components with wet hands. Do not place it in a humid environment such as an incubator. For more info, refer to Section Environmental Conditions.
2. Trypan Blue Stain, Acridine Orange Stain, Propidium Iodide Stain, and other reagents are known as hazardous materials. While handling the solution, always wear gloves to avoid exposure.
3. Before use, make sure that the input voltage is compatible with the instrument's power supply voltage.
4. For optimal operations, place the instrument on a flat bench 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 waste.
12. The LUNA-FL™ Automated Cell Counter is an electrical laboratory instrument for scientific research use only. It is not a medical, therapeutic, or in vitro diagnostics device.

<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-FL™ Automated Fluorescence Cell Counter

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

1. The instrument must be operated in compliance with the environmental conditions described on page 7. 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 slides by the edges to avoid touching the optical surface. Make sure that no damage or contamination occurs on the optical surfaces of the slide.
4. After mixing the cell sample with the supplied reagents, perform cell counting within 1-3 minutes for accurate cell viability measurements. Optimally, count your sample at least twice (duplicate readings) to obtain better results.
5. Since the LUNA-FL™ is calibrated before shipping, users generally do not need to re-calibrate before use. However, if re-calibration is needed, for example due to long-term use, please refer to Section 9.3 Calibrating the Counter.
6. Do not touch Trypan Blue Stain and other reagents with bare hands, as they are hazardous chemicals. After using slides, dispose of them as hazardous waste. Do not reuse the slides.

Environmental Conditions

Operating Power	100 - 240 VAC, 1.5A
Frequency	50/60 Hz
Electrical Input	12 VDC, 3.5A
Installation Site	Indoor use only
Operating Temperature	10 - 35℃
Maximum Relative Humidity	20 - 80%
Altitude	≤2,000 m
Pollution Degree	2

Chapter 1 – Introduction

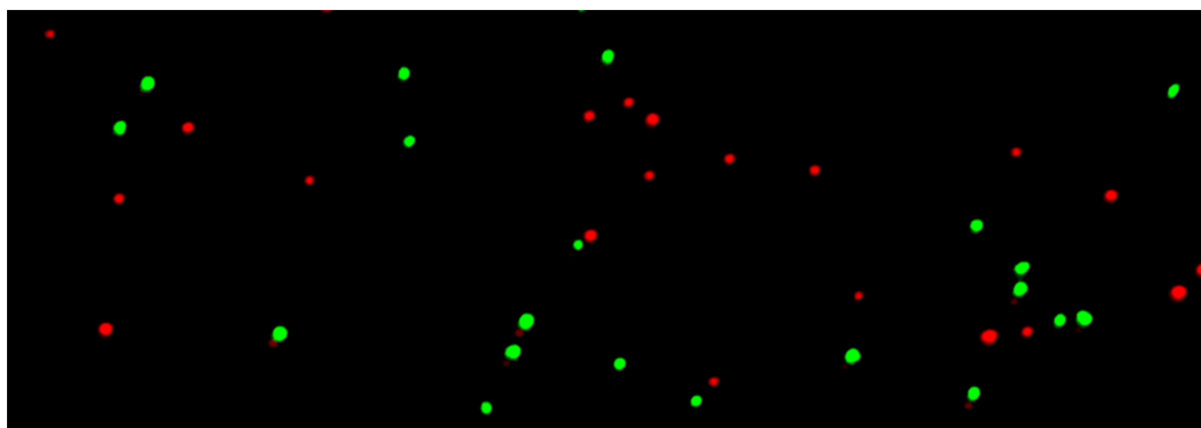
1.1 Product Overview

The LUNA-FL™ Automated Fluorescence Cell Counter is a small, fast, and affordable image-based cell counting device, containing bright field and dual fluorescence optics, that automatically counts various kinds of cells for research use.

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

The LUNA-FL™ can be used in a very simple procedure. For example, in order to accomplish dual fluorescence counting, first, mix 18 μl of the cell sample with 2 μl Acridine Orange/Propidium Iodide Stain (in case that there is not enough cell sample, 9 μl of the cell sample with 1 μl of the PhotonSlide™ or LUNA™ Reusable Slide can be used to load the total 10 μl in the counting chamber). Second, load 10-12 μl of the mixed cell suspension into a PhotonSlide™ or LUNA™ Reusable Slide. Third, insert the slide into the slide port of the instrument and adjust the focus knob to get an appropriate cell image. Last, press the "Count" button and the results of cell count and viability will be displayed on the screen. The counting image can be downloaded onto a USB drive in a TIF format for future analysis.

< Acridine Orange/Propidium Iodide Stained Cell Image>



Assay principle: AO stains all cells, and PI stains dead cells only. If AO and PI exist together, AO fluorescence is mostly quenched by PI. Therefore, AO fluorescence (green) is found in live cells only, and PI fluorescence (red) is found in dead cells only. Cell viability analysis using Acridine Orange/Propidium Iodide double staining is a proven and accepted method.

The LUNA-FL™ Automated Fluorescence Cell Counter provides the data below.

- Number of live and dead cells and their concentrations
- Number of total cells and their concentrations
- Viability percentage (% live cells to total cells)
- Cell images (showing live cells as green circles and dead cells as red circles)
- Histogram of cell size distribution and size gating
- Number & percentage of the GFP-transfected cells

The PhotonSlide™ is disposable and specifically designed for the LUNA-FL™. Each counting slide has two chambers, labeled A and B, one slide can be used for the same sample reading in duplicate or it can be used for two different samples.

Key features of the LUNA-FL™ are as follows:

Key features	Description
Bright field & dual fluorescence optics	The LUNA-FL™ integrates bright field, as well as dual fluorescence optical components to provide advanced cell counting functionalities. The cell image acquired from each channel (bright, green, and red) can be merged directly on the screen. The brightness of each color can be adjusted independently for accurate monitoring.
Small footprint	Due to its minimal size (22 cm x 21 x 9 cm) & weight (1.8 kg), it can be used on a clean bench or stored in a biosafety cabinet for convenience.
Accuracy & precision	Due to its sophisticated optical components and counting algorithm, LUNA-FL™ cell counter provides reproducible results every time.
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 7-30 seconds of pressing the 'Count' button.
Innovative counting slide	LUNA-FL™ cell counter 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 within the 1 x 10^4 to 1 x 10^7 (preferably 5 x 10^4 to 1 x 10^7) cells/ml

	range and for cells within the 1-90µm (preferably, 5-60µm) diameter range.
Cell size gating	After counting, users can set the gating parameter for cell size.
Easy-to-use dilution calculator	Dilution concentration can easily be calculated using the built-in calculator.
Set up & maintenance	Just plug in and it is ready for use, with virtually no maintenance time or costs.
Counting image transfer	The counting image can be downloaded onto a USB drive in TIF (Tag Image File) format for later use or review.
Individual protocol saving	Each user can set or adjust counting parameters and save the counting protocol for later use.
Automatic reporting	LUNA-FL™ cell counter provides a counting report based on the count results in PDF (Portable Document Format) immediately after performing a cell count.

1.2 Product Contents

The LUNA-FL™ Automated Fluorescence Cell Counter (Cat # L20001) includes the following components.

Component	Quantity
LUNA-FL™ Automated Fluorescence Cell Counter	1 unit
Power cord (including an adapter)	1 unit
PhotonSlide™	1 box of 50 slides
Acridine Orange/Propidium Iodide Stain	2 x 0.5 ml
Trypan Blue Stain, 0.4%	2 x 1 ml
LUNA™ Fluorescence Calibration Beads	0.5 x 1 ml
USB Drive, 16 GB	1 unit

Note: The PhotonSlide™ is a counting slide developed by Logos Biosystems that provides much better fluorescence signal, with ultra-low autofluorescence. Users of the LUNA-FL™ are recommended to use the PhotonSlide™, especially for the yeast counting and GFP transfection assay.

Note: When you receive the product package, please check that all the components listed above are included and that no damage has occurred during transit. The warranty does not cover damage that may occur during the shipping and handling process. Any damage claims must be filed with the carrier.

1.3 Product Specifications

1.3.1. LUNA-FL™ cell counter specifications

Instrument Type	Bench-top cell counter
Dimensions (WxDxH)	22 x 21 x 9 cm
Weight	1.8 kg (without the power cord/adapter)
Light Source	LED
Excitation Wave Length	470 +/- 20 nm (Blue)
Emission Wave Length	525 +/- 25 nm (Green), 600 LP (Red)
Cell Concentration Range	1 x 10^4 - 1 x 10^7 (optimally 5 x 10^4 - 1 x 10^7) cells/ml
Cell Diameter Range	1 - 90 (optimally 5 - 60) µm
Cell Circularity Range	30 - 100%
Cell Viability Range	0 - 100%
Image Resolution	CMOS camera (5 MP)
Image Type	TIF
Reporting	PDF report, .CSV file
LCD Display	7 inches (800 x 480 pixels)
Processing Time	Approx. 7 seconds for bright field & approx. 30 seconds for dual fluorescence (may depend on cell type and concentration)

1.3.2 PhotonSlide™ specifications

Material	Poly(methyl methacrylate) (PMMA)
Dimensions (WxDxH)	25 x 75 x 2.4 mm
Chamber Depth	100 µm
Chamber Volume	10 µl

1.3.3 LUNA-FL™ software (version 3.0)

- Dual fluorescence cell counting
- Bright field cell counting
- Yeast counting
- GFP transfection assay

1.4 Product Description

1.4.1 Front view of the LUNA-FL™ Automated Fluorescence Cell Counter

The front view of the LUNA-FL™ Automated Fluorescence Cell Counter shows a wide touch screen. This interface contains buttons for all functions and displays results.

<Front View>

1.4.2 Rear view of the LUNA-FL™ Automated Fluorescence Cell Counter

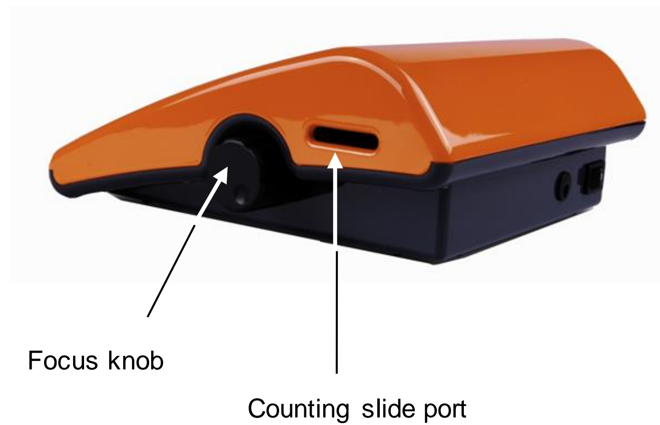
The rear view of the LUNA-FL™ Automated Fluorescence Cell Counter shows a power button to turn on or off the instrument and a power inlet. Connect the instrument to an electrical outlet with the power cord and plug provided in the product package. Be sure to check the electrical outlet configuration in your country.

<Rear View>

1.4.3 Right side view of the LUNA-FL™ Automated Fluorescence Cell Counter

The right side view of the LUNA-FL™ Automated Fluorescence Cell Counter shows a focus knob and a slide port. Use the focus knob to get optimized cell images by adjusting contrast between live cells with bright centers and dead cells with dark centers. The slide port is used to insert the slide loaded with the sample into the instrument.

<Right Side View>



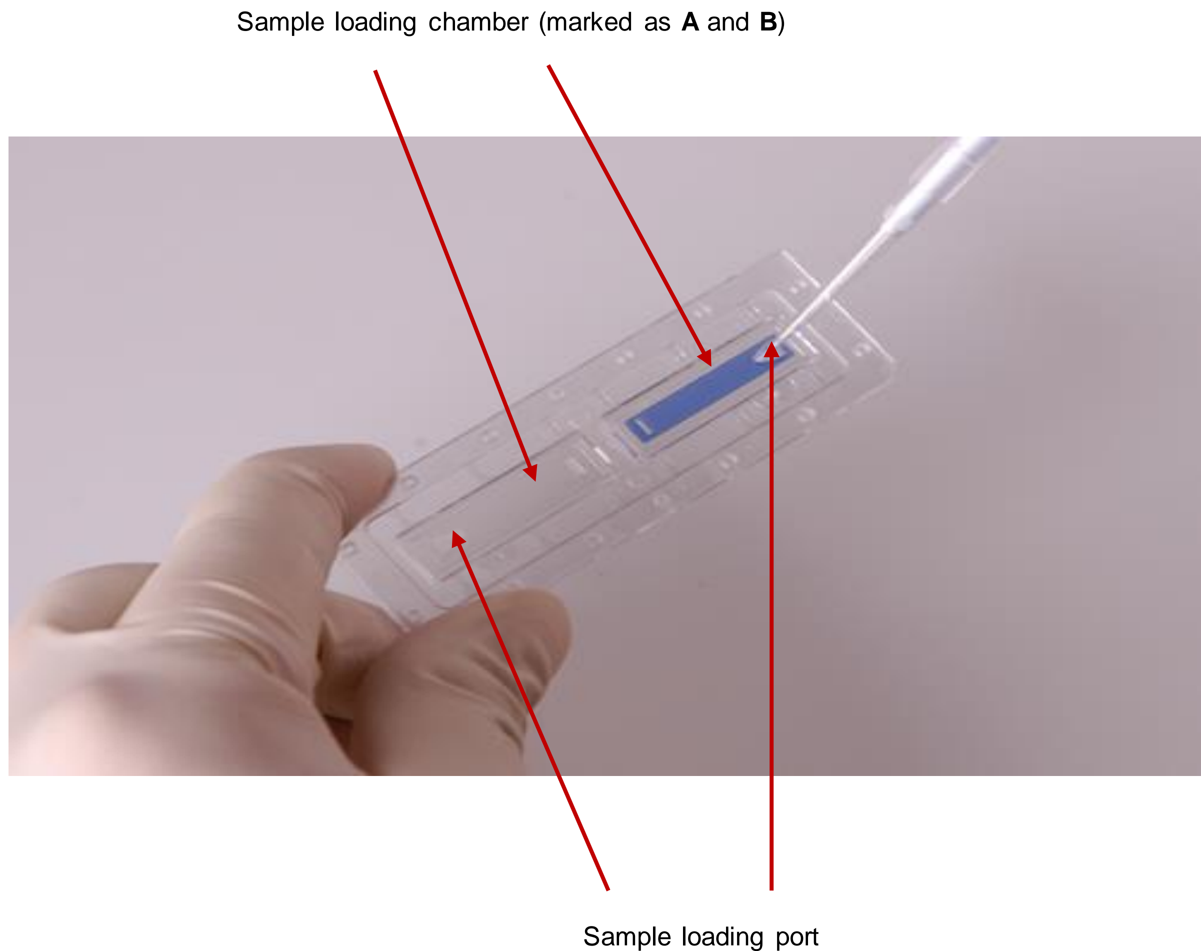
1.4.4 Left side view of the LUNA-FL™ Automated Fluorescence Cell Counter

On the left side of the cell counter, there is a USB drive port into which a USB drive is inserted for transferring and saving data. One USB drive is supplied in the product package. Any standard USB drive can be used.

<Left Side View>

1.4.5 The PhotonSlide™ for the LUNA-FL™

The PhotonSlide™ consists of two chambers, labeled as A and B, that can be used for the same sample as duplicates or for two different samples (for example, the GFP transfection assay). The depth of the counting chamber is 100 µm. The counted cell volume is about 0.5 µl, almost the same as five (1 mm x 1 mm) squares in a standard hemocytometer.



Note: 0.4% Trypan Blue Stain is blue and Acridine Orange/Propidium Iodide Stain is pink (or orange).

Chapter 2 – Setting up

2.1 Installation

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

2.1.2 Place the LUNA-FL™ Automated Fluorescence Cell Counter on a flat, stable surface.

2.1.3. 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.

2.1.4 Turn on the instrument using the power button located on the back of the cell counter.

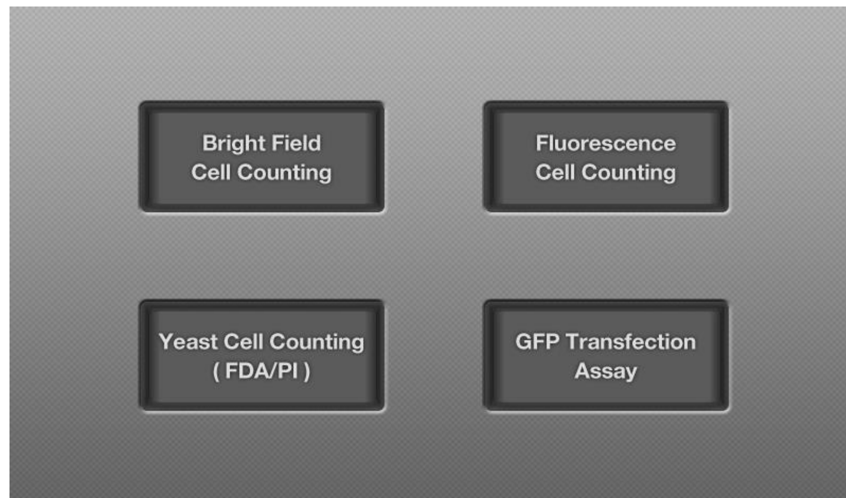
2.1.5 The Start-Up screen will display on the touch screen as shown below in 2.2.

2.2 Start-Up Screen

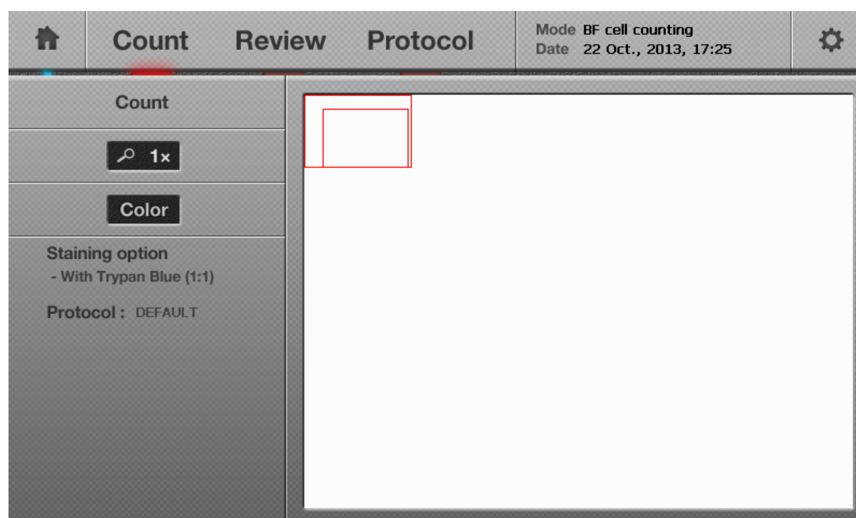
2.2.1 Home Screen

When the LUNA-FL™ is turned on, the Start-Up screen will display 4 options as shown below. Users can choose one of them to perform.

<Start-Up Screen of the LUNA-FL™ Automated Fluorescence Cell Counter>

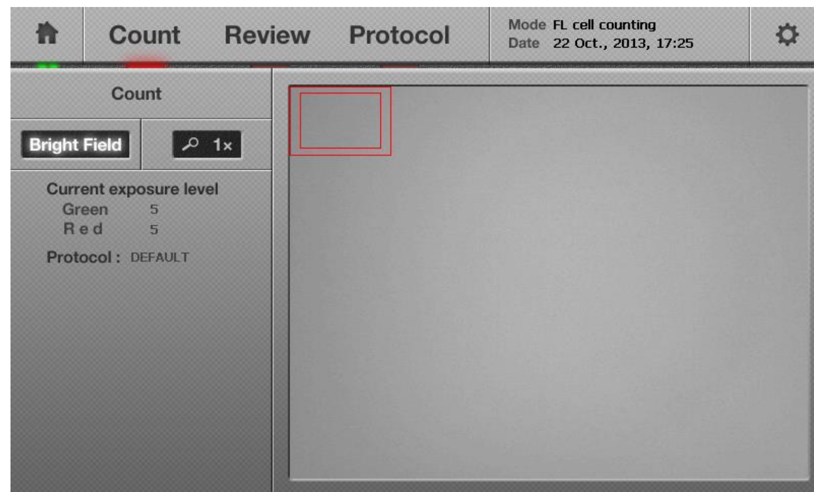


2.2.2 Bright Field Cell Counting Screen

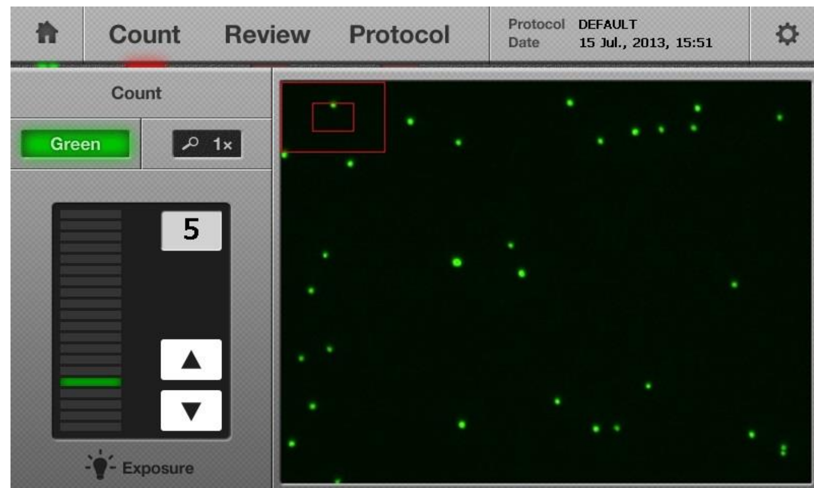


2.2.3 Fluorescence Cell Counting Screen

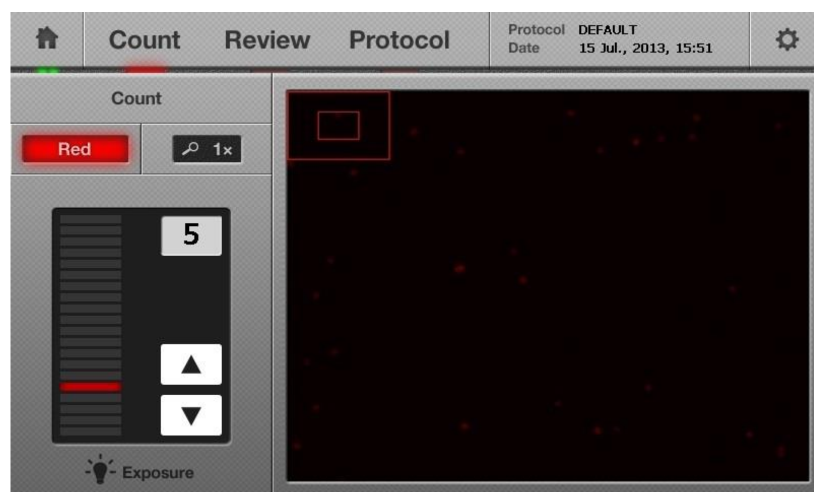
2.2.3.1 Fluorescence Counting – Bright Field Screen



2.2.3.2 Fluorescence Counting – Green Fluorescence Screen

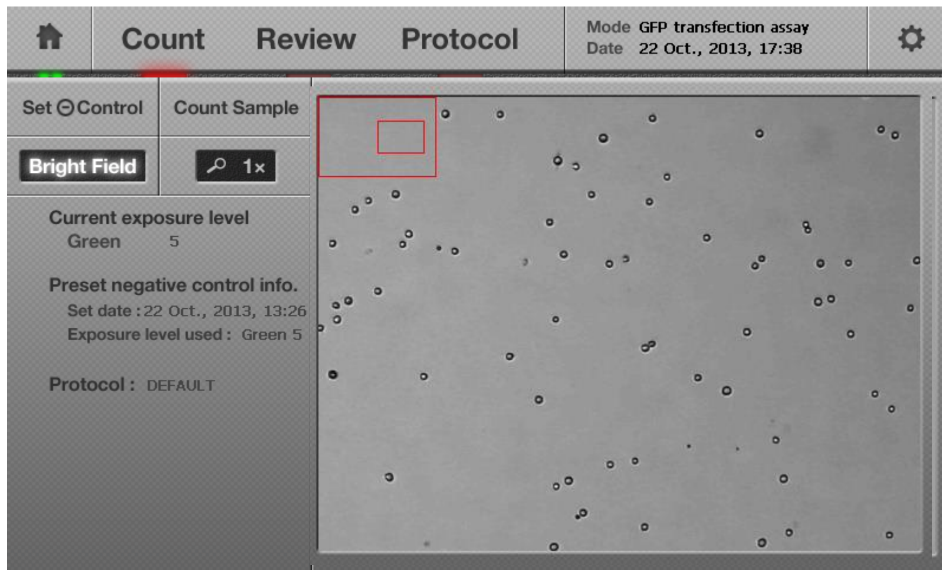


2.2.3.3 Fluorescence Counting – Red Fluorescence Screen

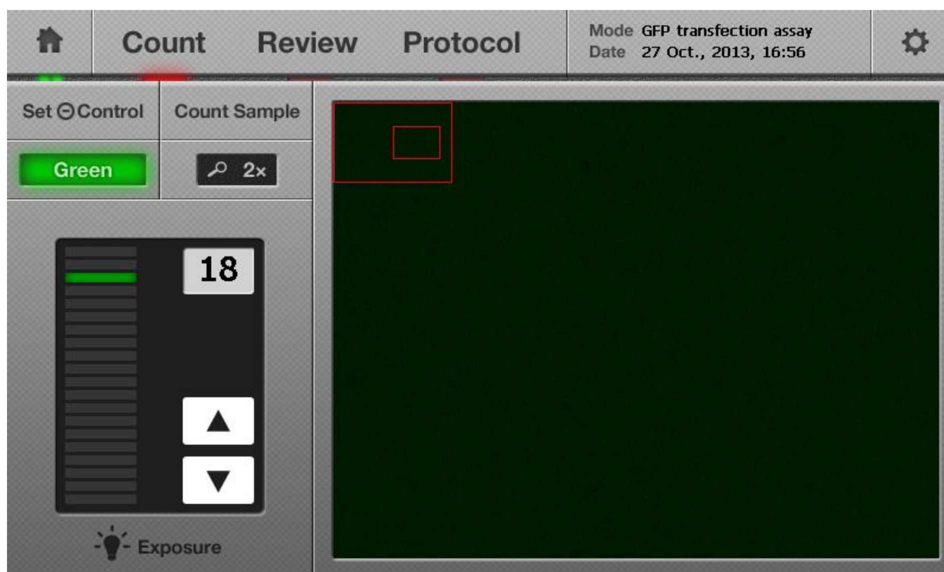


2.2.4 GFP Transfection Assay

2.2.4.1 GFP Transfection Assay – Bright Field Screen

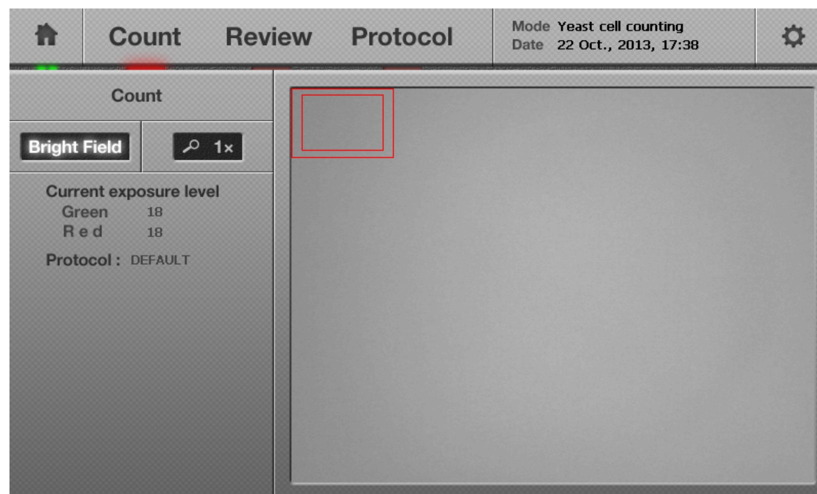


2.2.4.2 GFP Transfection Assay – Green Fluorescence Screen

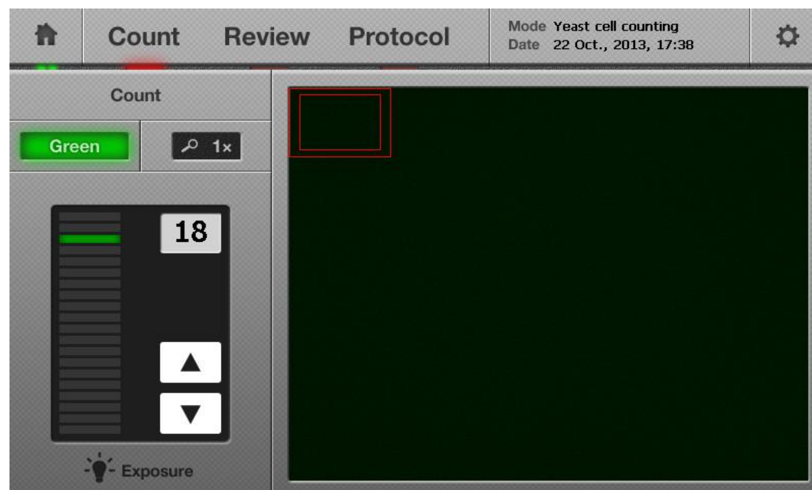


2.2.5 Yeast Cell Counting Screen

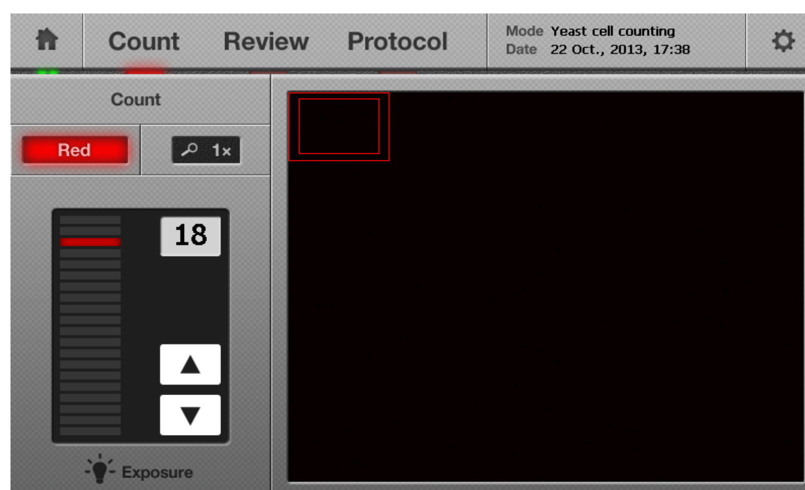
2.2.5.1 Yeast Cell Counting – Bright Field Screen



2.2.5.2 Yeast Cell Counting – Green Fluorescence Screen



2.2.5.3 Yeast Cell Counting – Red Fluorescence Screen



2.3 Settings

Generally, users do not 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 from the “Settings” menu described below.

2.3.1 After turning on the instrument, go to one of the 4 counting screen options.

2.3.2 Press the “Settings” button ( ) located on the upper right corner of the counting screen. Then, the following “Settings” screen appears. From here, users can change or adjust various preferences or options.

<Settings>

Note: Please make sure that the software version is 3.0.0 or higher. If necessary, download the latest software from the web site (www.logosbio.com).

2.3.3 The “Settings” menu allows you to perform the following:

- Background calibration of the instrument (described in Section 9.3 Calibrating the Counter)
- Firmware update for the installation of new versions of the firmware that will be released from time to time (described in Section 9.4 Updating the Firmware)
- Date and time set up
- Other options such as “Save”, “Counting”, and “Result/Printer/Calculator”

2.4 Setting the Date and Time

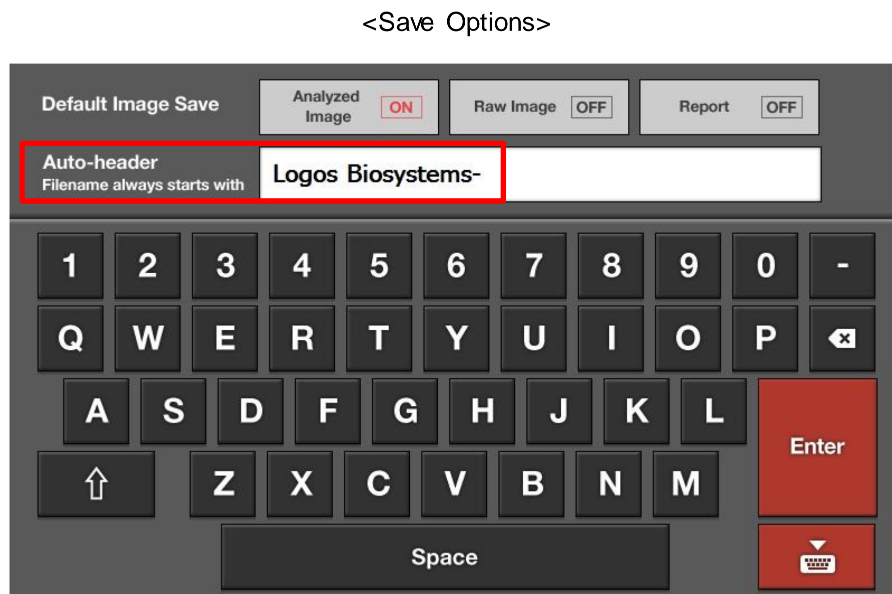
2.4.1 On the “Settings” screen, select the date and time buttons.

2.4.2 After selecting the field, erase the number using the “Backspace” button (⌫), set the date or time using the number buttons, and press the “Apply” button (Apply) to save the changes. .

2.5 Save Options

2.5.1 The “Save Options” screen will be seen as below, after selecting the “Save Options” button. As a default, only the “Analyzed Image” button is activated (On), while the “Raw Image” and “Report” button are inactivated (Off).

<Save Options>



2.5.2 In the “Default Image Save” menu, users can choose their options to save the raw image, PDF report, or both. Each button can be switched on and off.

Saved Items	Description
Analyzed image	The image contains the counting results and the tagging of live and dead cells.
Raw image	The image contains only the captured image for counting. In the bright field cell counting mode, the bright field image will be displayed. In the fluorescence cell counting, bright field, green, and red images will be

	displayed.
Report	The PDF report containing counting results and histograms

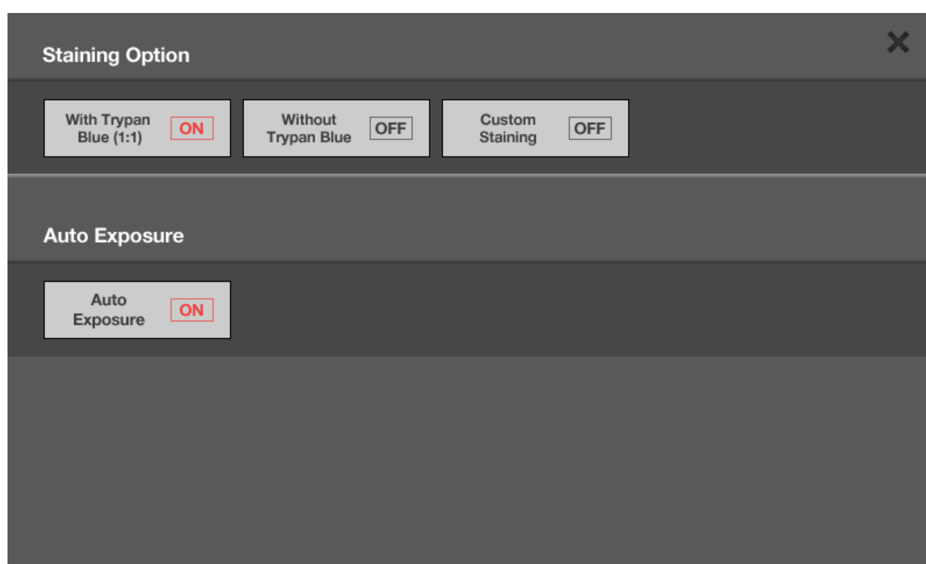
Note: Remember that the “Raw Image” must be turned on to save and send the original cell images for getting the best technical support.

2.5.3 The “Auto-header” which is red-lined in the above “Save Options” image is the newly created folder name or file name that all the images start with during the saving process. After saving, users can see a folder or a file name, containing the saved item, which begins with “Auto-header” in the root directory of the USB drive. In case users want to have another default name, the “Auto-header” can be customized by editing the default value of “Logos Biosystems-”.

2.6 Counting Options

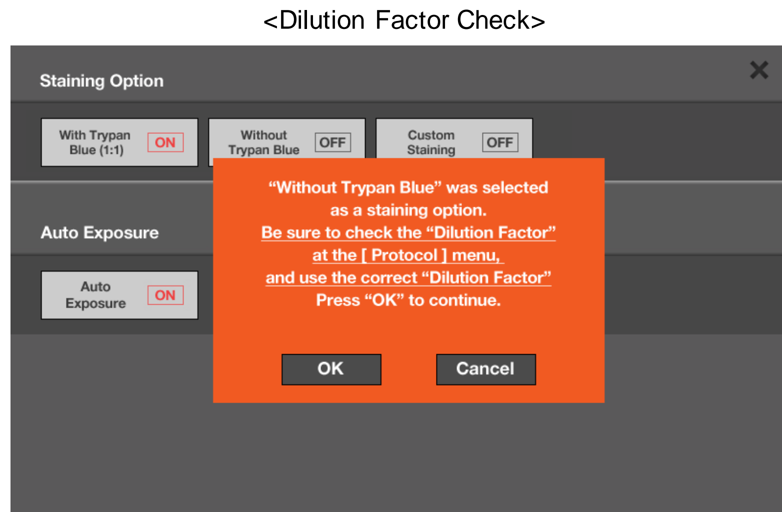
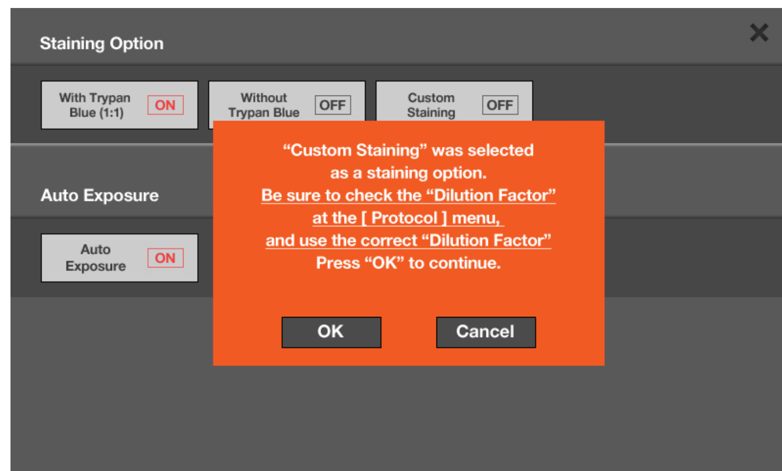
2.6.1 The “Counting Options” applies only to the bright field cell counting. Before selecting an option, please make sure that the “Bright Field Cell Counting” is selected from the options on the Home Screen.

<Counting Options>



2.6.2 In the “Staining options” of “Counting Options”, users can select one of the three staining options as below.

Staining Options	Description
With Trypan Blue (1:1)	This option can be used for normal bright field counting, when cell samples are mixed with Trypan Blue dyes in a 1:1 ratio. This option can generate cell viability data.
Without Trypan Blue	When samples do not contain Trypan Blue dye, turn on this button and follow the directions in the message boxes. These instructions set the “Dilution Factor”. For samples with Trypan Blue dye, the dilution factor must be set to value “2”. For samples without Trypan Blue dye, the dilution factor must be set to value “1”. **Note**: The cell counting parameters of the “Bright Field Cell Counting” mode of the LUNA-FL™ is optimized with the use of Trypan Blue. Although the counting “Without Trypan Blue” option is provided in the protocol menu to meet mostly the “total cell counting” for some special users, it is **NOT** recommended to count cells without Trypan Blue staining. Low contrast due to the lack of the Trypan Blue staining may sometimes cause abnormal

	results. <Dilution Factor Check>  **Note**: The setting of the "Dilution Factor" must be checked and done manually.
Custom staining	In this option, cell samples can be stained with Erythrosin B Stain or other dyes (or even the culture media) for counting. Before choosing this option, the instrument must be re-calibrated with users' own stains or culture media. For re-calibration, please refer to Section 7.3 Calibrating the Counter. When using this option, make sure that the dilution is 1:1 (cell sample vs. staining solution) and the "Dilution Factor" must be set to value 2. 

2.6.3 “Auto Exposure” function can be only applied using “With Trypan Blue (1:1)” options. As a default, “Auto Exposure” button is activated (On).

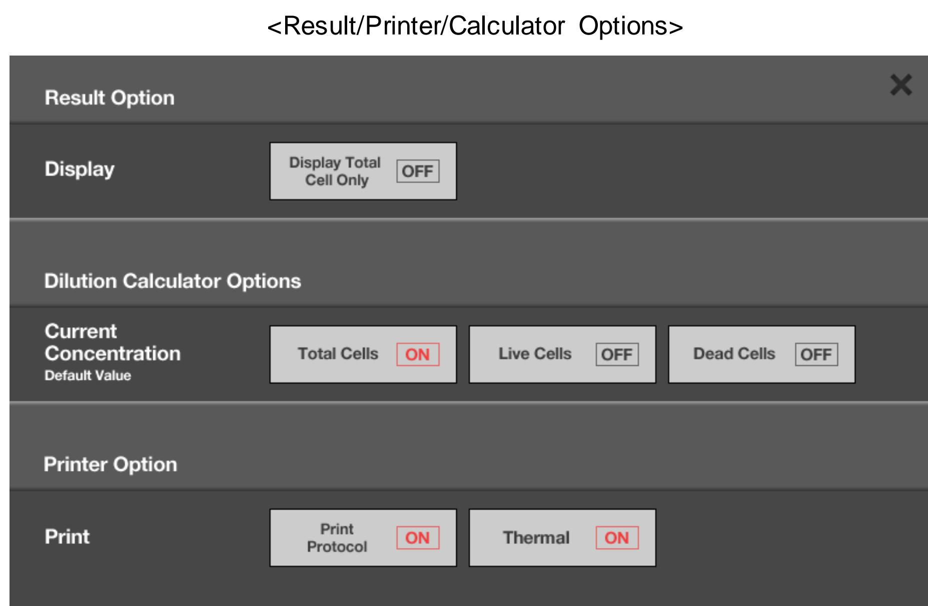
The description of “Auto Exposure” option is as below

Auto Exposure	Description
ON	Users use this option to minimize deviation of counting results according to concentration of the reagent on “With Trypan Blue (1:1)” mode. As “Auto Exposure” is on, background of captured image is adjusted automatically even though background of sample is a little brighter or darker. **Note**: The LUNA-FL™ basically analyze results based on image, so the instrument can provide an accurate counting results when staining cells with appropriate 0.4% Trypan Blue Stain. Because of that, deviation in concentration of stained sample may can cause unexpected results if concentration of reagents changes for any other reason. In these case, users can minimize effects of background deviation that may cause counting results by using Auto Exposure option.
OFF	As “Auto Exposure” is off, samples are captured and counted by set value of background calibration that is made previously.

2.7 Result/Printer/Calculator Options

2.7.1 This option shows three different menus, as below.

<Result/Printer/Calculator Options>



Option	Sub-option	Description
Result	Display	The default value is "off." When it is turned on, the counting result box displays only the total cell number, not showing live/dead cells.
Dilution Calculator	Current Concentration	The default value is "Total Cells (on)." In order to use the "live cells" in the Dilution Calculator, please turn the "live cells" on.
Printer	Print Protocol	When this button is on, the print-out of the results has the details of the parameters of counting. Otherwise, the part of the counting parameters will not be printed.
	Thermal	Currently, the default printing paper is a thermal printing roll (A label printing paper will be available later). Make sure that "Thermal" is on.

Note: Depending on the 4 counting modes, one or more options are not available or inactivated.

Chapter 3 – Bright Field Cell Counting

3.1 Sample Preparation

3.1.1 Prepare the following materials in order to count cells.

- Cell sample
- PhotonSlide™ (Cat # L12005)
- Trypan Blue Stain, 0.4% (Cat # T13001)

3.1.2 If desired, insert a USB drive to save data and results.

3.1.3 Mix 10 µl of the cell sample with 10 µl 0.4% Trypan Blue Stain gently in a microfuge tube by pipetting up and down.

Note: The cell counting parameters of the "Bright Field Cell Counting" mode of the LUNA-FL™ is optimized with the use of Trypan Blue Stain. Although the counting "Without Trypan Blue" option is provided in the protocol menu (section 2.6.2) to meet mostly the "total cell counting" for some special users, it is **NOT** recommended to count cells without Trypan Blue staining. Low contrast due to the lack of the Trypan Blue staining may sometimes cause abnormal results.

Note: Erythrosin B Stain for an alternative of Trypan Blue Stain also can be used. Erythrosin B is less toxic to your cells. Stained cell with Erythrosin B must be counted with "Custom staining" option of "Bright Field Cell Counting" mode.

3.2 Loading Samples into Slides

3.2.1 Holding the edge of the slide, load 10-12 µl of the mixed cell sample into the inlet of one chamber of the counting slide. For easy and accurate loading, tilt the pipette around 45-60 degrees as shown below.

<Sample Loading>

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

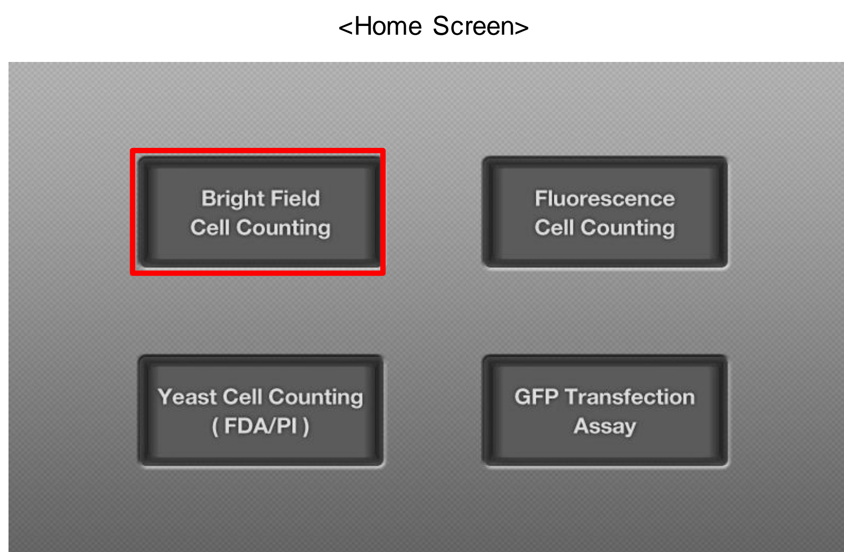
3.3 Counting Cells

3.3.1 Insert the loaded slide into the slide port of the instrument, ensuring that the loaded chamber is inserted first. The instrument analyzes only the first inserted chamber. Gently insert the counting slide to the end.

Note: After inserting the slide, the LUNA-FL™ only reads the first chamber. To read the second chamber, it must be taken out, rotated, and inserted again. Make sure that the counting slide is not inserted upside-down. This may lead to sample spilling and could severely damage the counter.

3.3.2 Select the “Bright Field Cell Counting” button on the home screen.

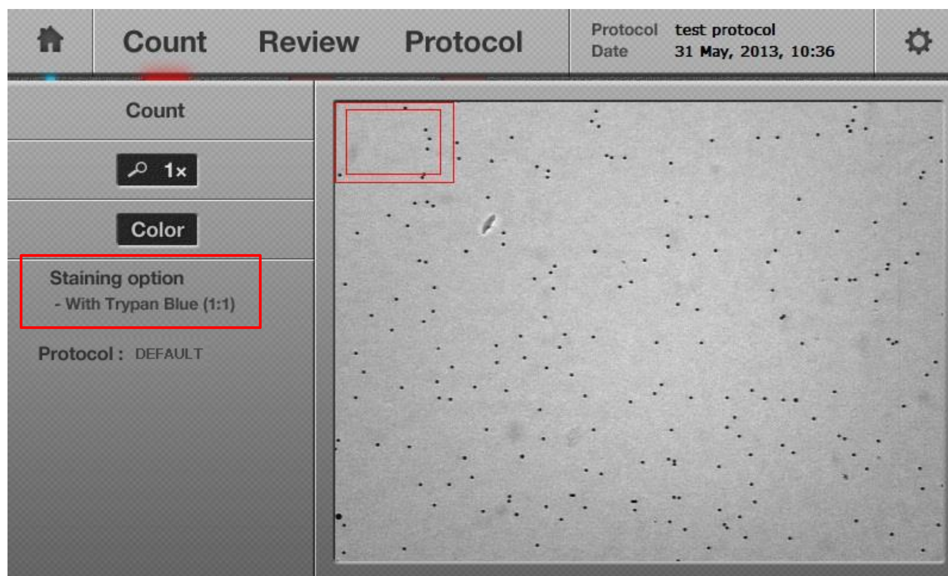
<Home Screen>



Note: After this step, the real image of the cell sample will be displayed on the screen. The cell image can be navigated by touching the screen and moving the finger or stylus pen on the image. Please note that the touch screen of the LUNA-FL™ Automated Fluorescence Cell Counter is a resistive touch screen, which needs a bit of pressure in order to get a response.

3.3.3 The “Preview screen” will appear as shown in the image below. When necessary, adjust the focus with the focus knob on the right side of the instrument to make sure that live cells have bright centers and dark edges, and dead cells have uniform dark color all over them.

<Preview Screen>

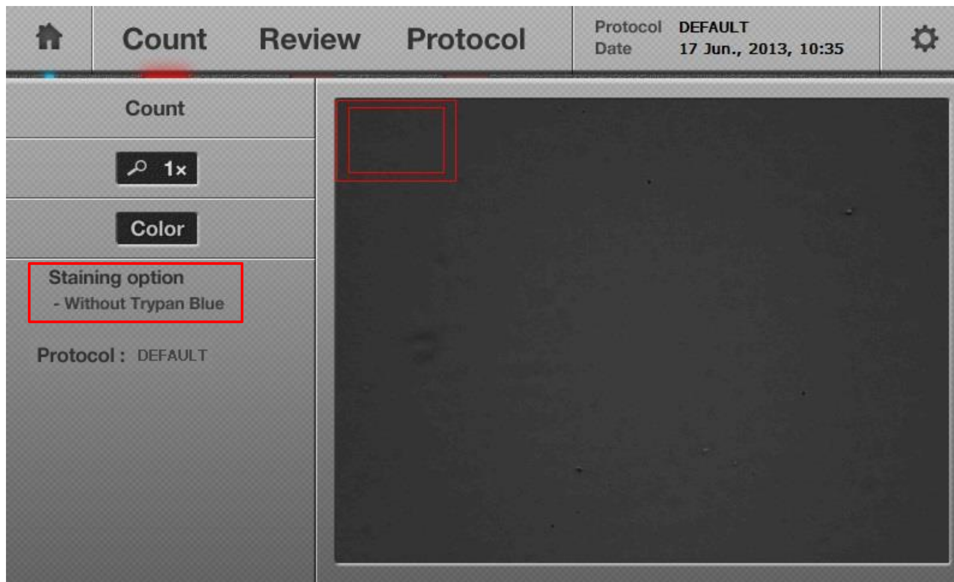


Note: Users must find an accurate focus to get the best cell counting results.

Note: To get the best focus, the image can be magnified by using the “Zoom-in” button located under the “Count” button. Initially, the “Zoom-in” button is set to a 1x image. When pressed once, a 2x image appears. This 2x image may be the best zoom-in status for focusing. When pressed again, a 4x image is shown. Pressing the button again takes the screen back to a 1x image.

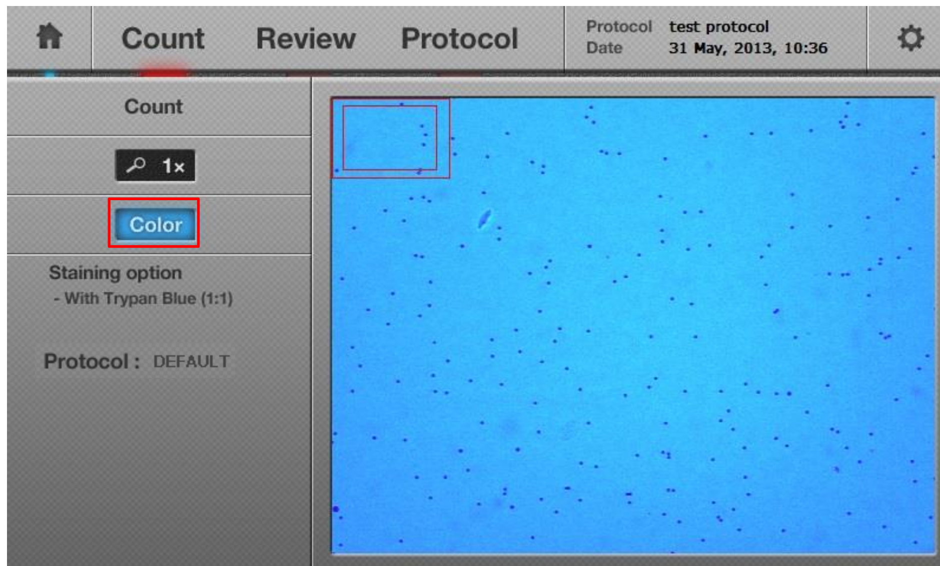
Note: The current staining option is displayed on the left side of the screen as marked by the red box in the image above. The staining option can be changed in the settings menu (section 2.6.2). Selecting the wrong staining option will result in an under-exposed preview image as shown below in which the “Without Trypan Blue” option is selected while Trypan Blue is present.

<Wrong Staining Option Selected: Too Dark Preview Image>



Note: The default preview image is set to a grey scale. To change the preview image to a color mode, press the "Color" button once, and the preview image will turn to a color image and the "Color button" will change to bright blue. Regardless of which preview mode is selected, grey or color, grey images will be used for calculating the cell number displayed in the cell counting result screen.

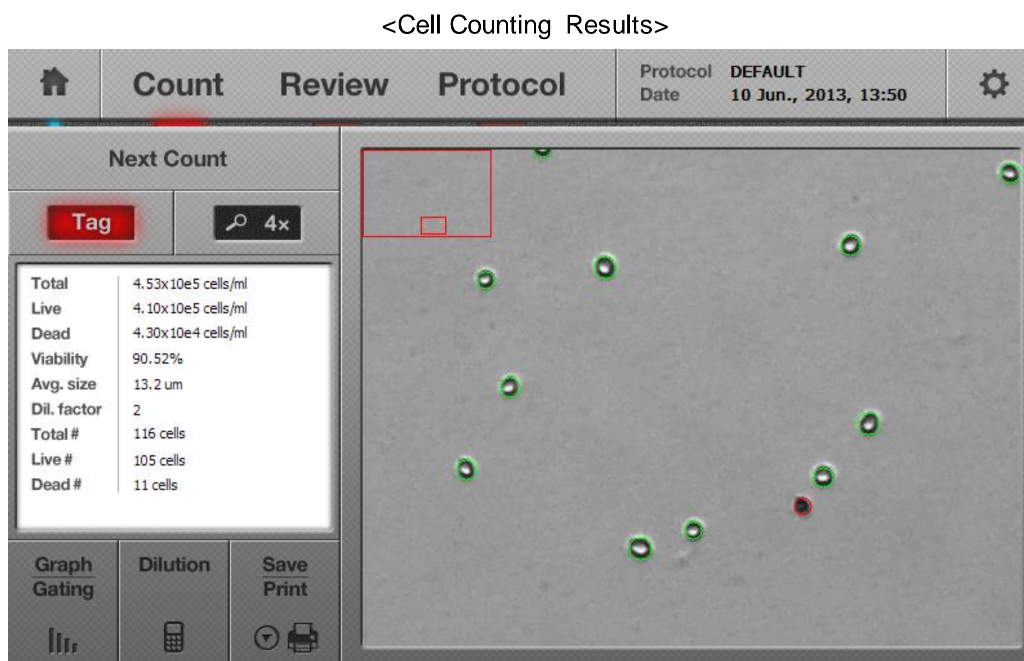
<Color Preview Screen>



3.3.4 Press the “Count” button, which is located on top of the “Zoom-in” button.

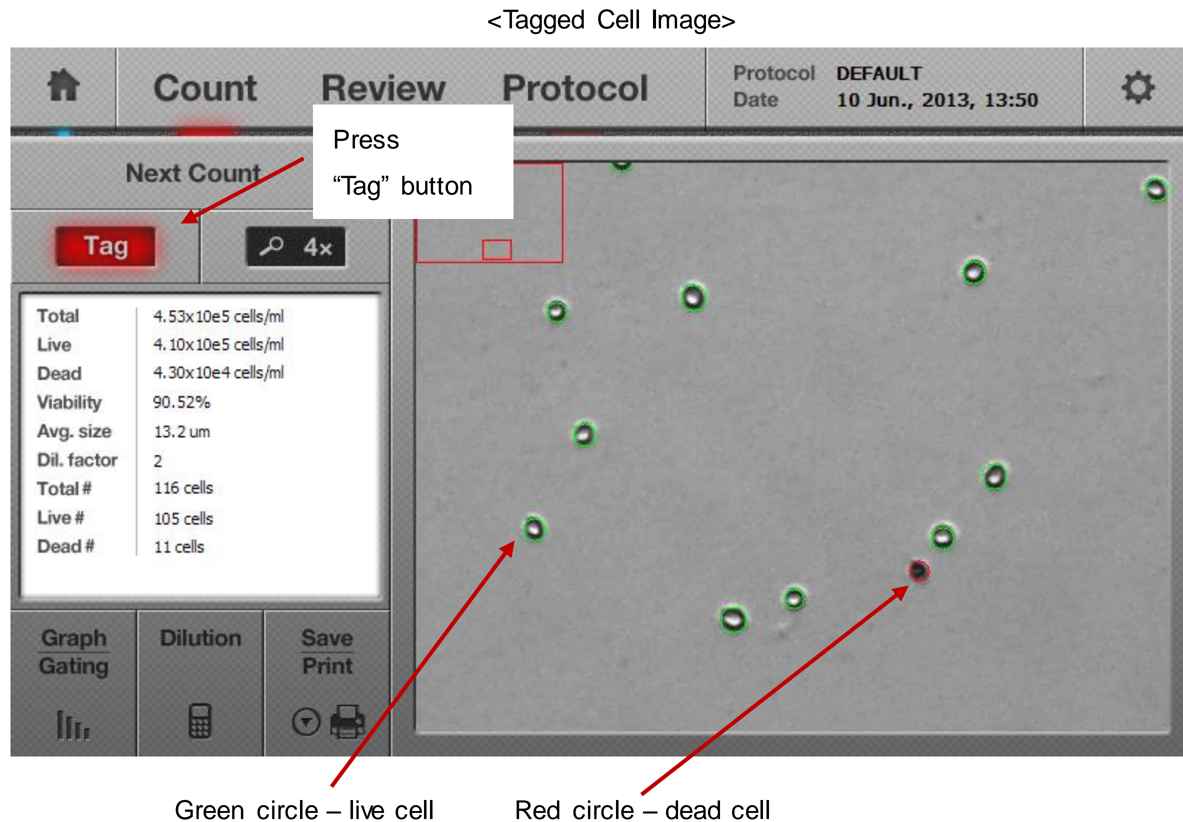
3.3.5 Within approximately 7 seconds (depending on cell concentration), the image of the cell sample and data/results (Total, Live, and Dead cell concentrations, Viability, Avg. Cell Size, Actual number of Total, Live and Dead cells) will be displayed on the screen.

<Cell Counting Results>



3.4 Using the “Tag” Function

3.4.1 After performing the counting function, the “Tag” button can be used to verify the counting results.



Note: This “Tag” function is one of the distinct tools of the LUNA-FL™ Automated Fluorescence Cell Counter because it allows the user to review the data to determine the accuracy of the counting on-site without depending on a computer.

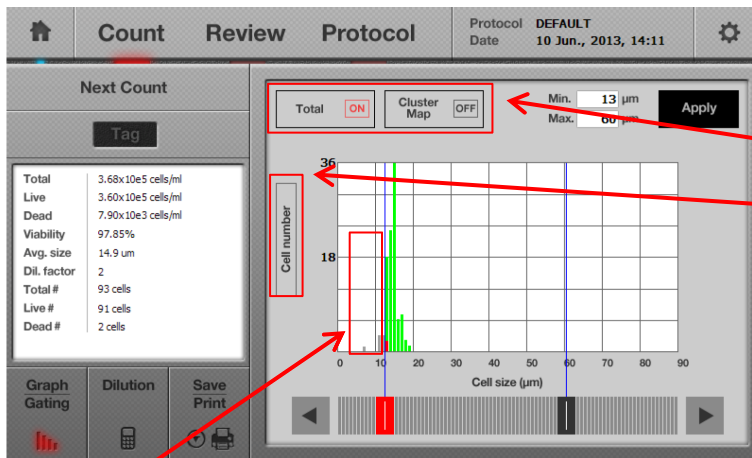
3.4.2 When touching the “Tag” button, the image on the screen will show objects surrounded by green or red circles. The green circles indicate live cells and the red circles indicate dead cells.

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

3.5 Cell Size and Number Distribution

3.5.1 To obtain more data on the distribution of cell size and number in graphical representation, press the “Graph/Gating” button on the lower left side of the screen. A histogram will appear with more details on the size and number of the cell sample as shown below. Live cells are displayed as a green bar and dead cells are displayed as a red bar.

<Graphical Representation of Cell Size Distribution>



Functional buttons in the menu

“Count → Graph/Gating”

1) “Total, Live or Dead” button

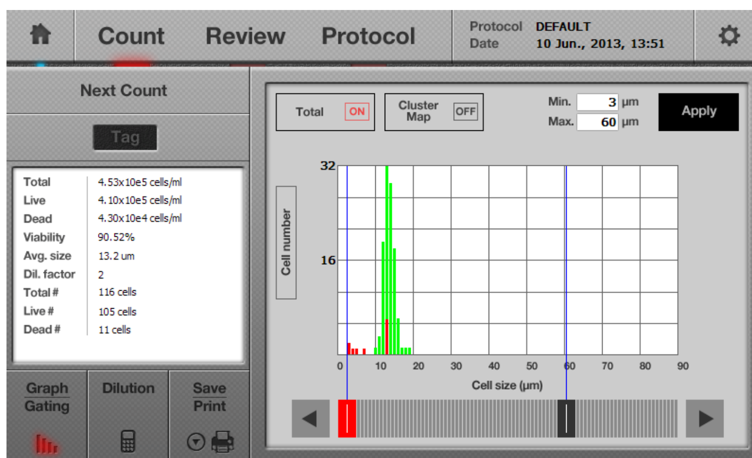
2) “Cluster Map” button

3) “Cell number or /ml” button

* The above buttons can be used in combination and the details are described in next pages.

When the sizes of the cells are out of the range, they are displayed in grey color.

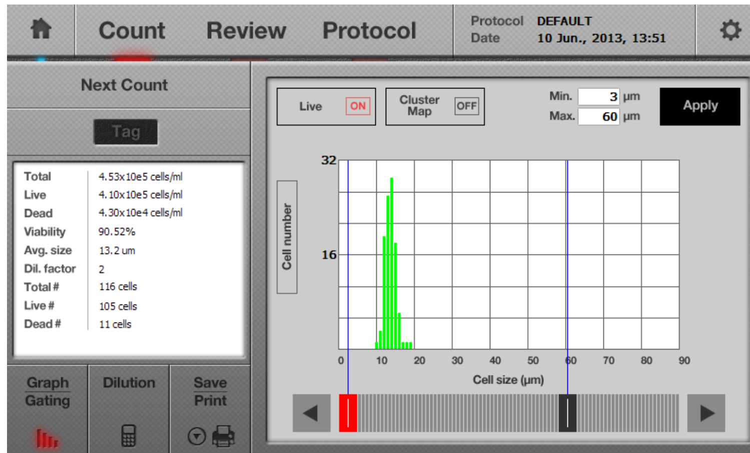
<Combination: Total and Cell Number>



Combination of “Total” and “Cell number” shows the distribution of cell size vs. cell number for total cells.

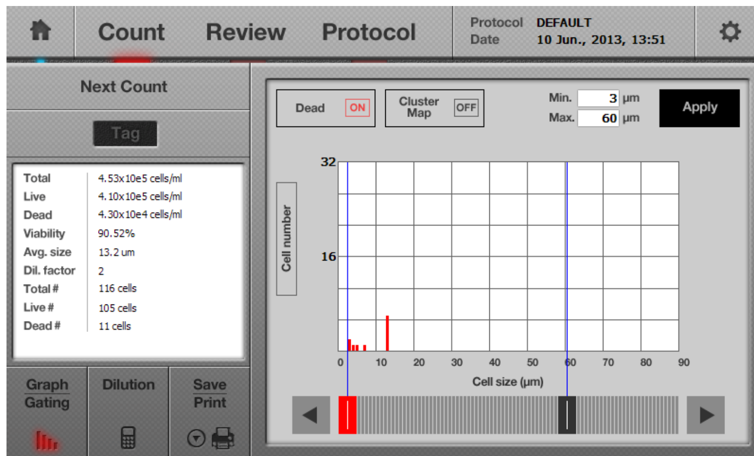
Note: When the “Total” button is pressed, it toggles to the “Live” button, which shows only live cells as shown below. When the button is pressed again, it toggles to the “Dead” button to show only dead cells as in the next figure.

<Combination: Live Cells and Cell Number>



The combination of "Live" and "Cell number" shows the distribution of cell size vs. cell number for only live cells.

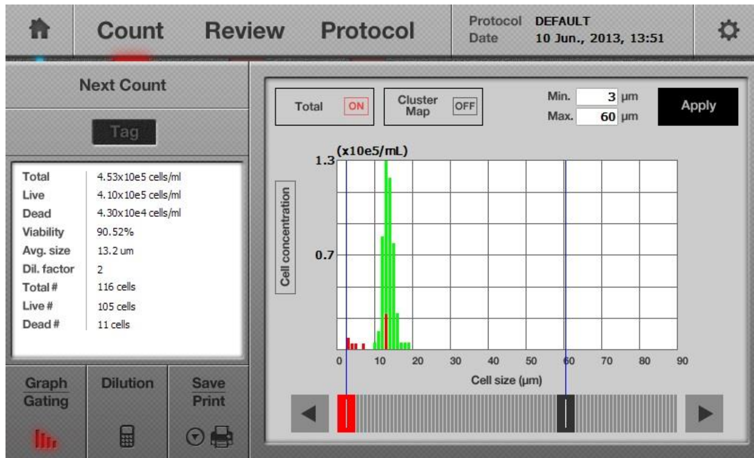
<Combination: Dead Cells and Cell Number>



The combination of "Dead" and "Cell number" shows the distribution of cell size vs. cell number for only dead cells.

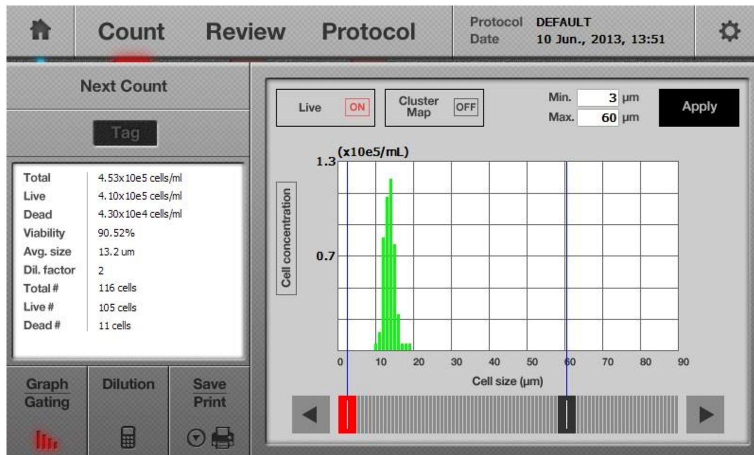
Note: When the "Cell Number" button is pressed, it toggles to the "/ml" button which shows the cell concentration (i.e., the Y axis of the histogram). When pressed again, it toggles to the "Cell Number" button.

<Combination: Total Cells and Concentration>



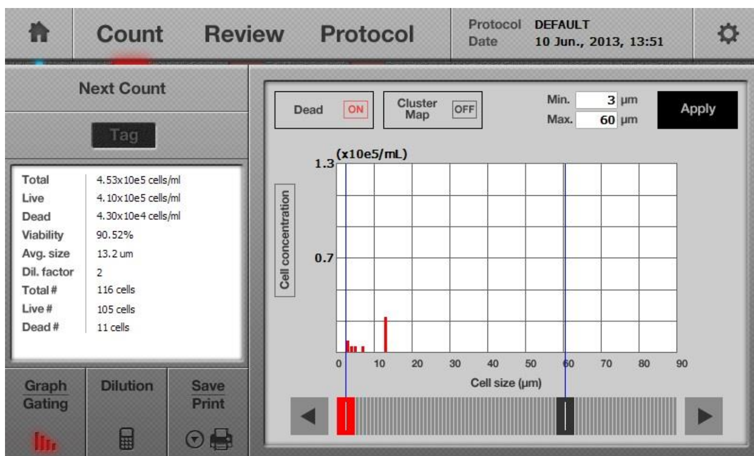
The combination of “Total” vs. “Cell concentration” shows the distribution of cell size vs. cell concentration for total cells.

<Combination: Live Cells and Concentration>



The combination of “Live” vs. “Cell concentration” shows the distribution of cell size vs. cell concentration for only live cells.

<Combination: Dead Cells and Concentration>

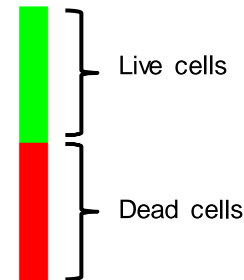


The combination of “Dead” vs. “Cell concentration” shows the distribution of cell size vs. cell concentration for dead cells.

3.5.1.1 Graphical representation of live and dead cells

When the “Total” button icon is clicked, the histogram is expressed as a “Stacked Column Chart.”

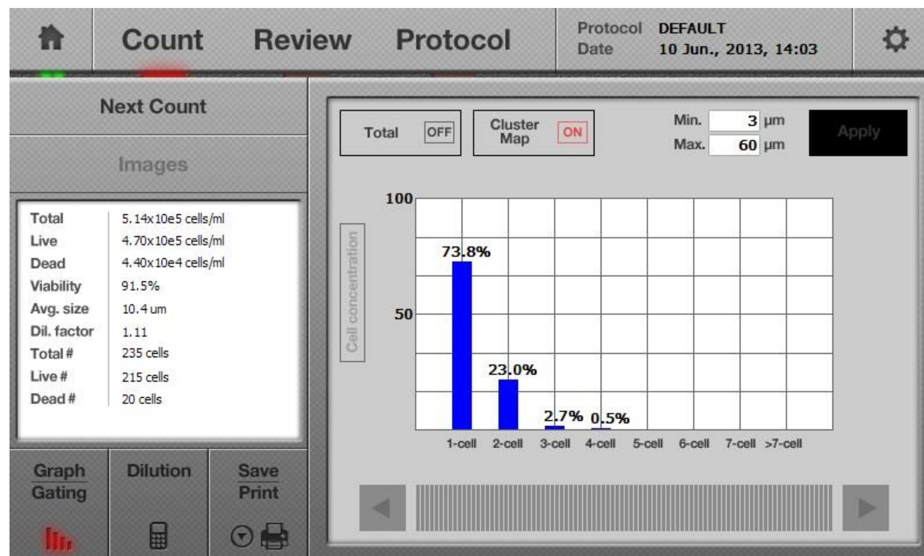
<Graphical Representation of Live and Dead Cells>



3.5.2 “Cluster Map” button

3.5.2.1 When the “Cluster Map” button is pressed, the percentage distribution of the clustered cells are displayed. “1-cell” means single cell and “2-cell” means 2 clustered cells as a unit. When the “Cluster Map” button is on, the “Total” and “Cell Number” buttons become inactivated.

<Cluster map>



3.5.2.2 Press the “Graph/Gating” button again to go back to the previous screen.

3.6 Using the Dilution Calculator

3.6.1 The LUNA-FL™ Automated Fluorescence Cell Counter provides a built-in dilution calculator that helps easily calculate adjustments to obtain a desired concentration.

<Built-in Dilution Calculator>

3.6.2 The dilution calculator initially shows the current concentration. Put the appropriate numbers into the blanks of the “Desired Concentration” and “Final Volume” that you want to obtain.

Users can choose one of “Total”, “Live” or “Dead” cells for calculating their dilution. By clicking this button, the “Current Concentration” displays the concentration of the total, live or dead cells. Before calculating the dilution factor, please check this option to match your requirements.

Users can also calculate their custom dilution.

3.6.3 Press the “Calculate” button and then a dilution instruction will appear in the message box.

3.7 Saving Images and Generating a PDF Report of the Current Count

3.7.1 To save data and/or generate a report, insert a USB drive into the USB port on the left side of the instrument.

3.7.2 Press the "Save" button located on the lower left part of the screen and a new "Save" window will pop up on the screen as shown below.

<Save and Report Screen>

Note: This "Save" window contains three check boxes as follows:

- Analyzed Image: Check this box to save an analyzed image. Live cells are tagged in green and dead cells are tagged in red for further analysis. If not checked, users can not use the "Review" function with the saved image. So, please make sure that this must be checked if users want to review the saved image on the instrument later.
- Raw Image: This box must always be checked in order to save the image in TIF format. The saved image can be re-analyzed on the instrument later if necessary.
- Report: Check this box to generate a printed report as a PDF. This report contains all the information of the cell counting session. This report can be read and printed with a personal computer (PC). Please un-check if a report is not necessary.

Note: Remember to save and send the "Raw Image" for getting technical support from distributor or manufacturer.

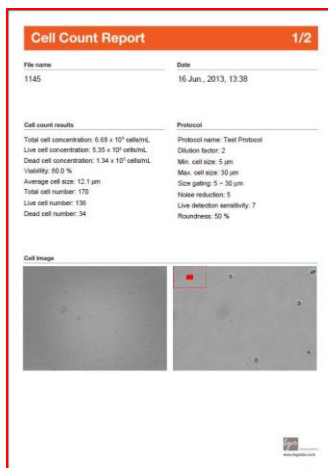
3.7.3 Enter the file name using the keyboard buttons on the screen. The date and time can be added by pressing the “Date/Time” button.

Note: Make sure that the cursor is located in the file name box before entering the file name.

3.7.4 Once the file name is verified, press the “Enter” button to save the image and/or report into the USB drive. The saved files can be opened after transferring the images/report via USB drive to a PC with the appropriate software.

Note: With the LUNA-FL™ software, additional graphical representations of the data and results are provided in the PDF report, as shown below.

<Two page PDF report generated by LUNA-FL>


Cell Count Report 1/2

File name: 1145

Date: 16 Jun., 2013, 13:38

Cell count results

Total cell concentration: 6.68 x 10[illegible] cells/mL
Live cell concentration: 5.35 x 10[illegible] cells/mL
Dead cell concentration: 1.34 x 10[illegible] cells/mL
Viability: 80.0 %
Average cell size: 12.1 µm
Total cell number: 170
Live cell number: 136
Dead cell number: 34

Protocol

Protocol name: Test Protocol
Dilution factor: 2
Min. cell size: 5 µm
Max. cell size: 30 µm
Size gating: 5 ~ 30 µm
Noise reduction: 5
Live detection sensitivity: 7
Roundness: 50 %

Cell Image

<1st page - Data and Results>

Cell counting data

Protocol used

Cell images (tagged and zoomed)

Cell Count Report 2/2

Cell size histogram expressed by cell number

Total cell | Live cell | Dead cell

Cell size histogram expressed by cell concentration

Total cell | Live cell | Dead cell

Cell cluster graph

<2nd page - Analytical Histograms>

By cell number (Total/Live/Dead)

By cell concentration (Total/Live/Dead)

By cell clustering

3.8 Printing the Counting Results

3.8.1 Users can print the counting results with the LUNA™ Printer (Cat # P10001) and LUNA™ Printer Paper (Cat # P12001). The LUNA™ Printer is a mobile printer, which can be charged with the battery pack before use.

<LUNA™ Printer>

USB Connection Port

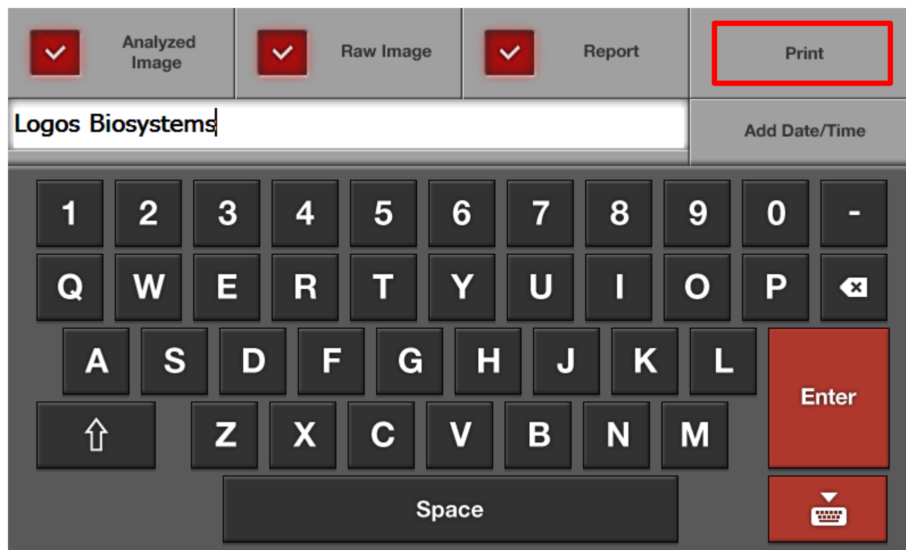
Note: The LUNA™ Printer and Paper are specially designed for the LUNA-FL™ and must be purchased only from Logos Biosystems or its local distributors.

Note: For more information on the LUNA™ Printer, please see the User Manual of the LUNA™ Printer.

3.8.2 First, connect the LUNA™ Printer to the LUNA-FL™ via the supplied USB connector (The LUNA™ Printer can be connected at any time.) Then turn on the LUNA™ Printer by pressing the “Power” button for 1-2 sec (To turn off, press the “Power” button for 2-3 sec.).

3.8.3 Go to the “Save and Report” screen and then click the “Print” button.

<Print Button>



3.8.4 The counting results and its protocol will be printed out as below.

<Printed Results>

```
  Cell Count Report
================================
Instrument: LUNA-FL Automated Cell Counter
File name: Logos Biosystems-
Date: 15 Jun., 2013, 11:40
Cell Counting Mode: Fluorescence

  Cell count results
  -------------------
[Total cell]: 3.88x10e5 cells/mL
[Live cell]: 3.88x10e5 cells/mL
[Dead cell]: 0.00x10e0 cells/mL
Viability: 100.0%
AvgSize: 14.1 um
Dil.Factor: 1.11
Total cell: 178 cells
Live cell: 178 cells
Dead cell: 0 cells

  Protocol
  ---------
Protocol name: DEFAULT
Min. cell size: 5 um
Max. cell size: 60 um
Size gating: 5~60 um
Green Threshold: 3
Red Threshold: 3
================================
```

Note: The “Print” button prints only the latest counting results.

Note: If the “Protocol” part of the print is not necessary, users can turn off the option in the “Results/Printer/Calculator” Option in the “Settings” menu, as shown below.

<Result/Printer/Calculator Options>

Result Option

Display — Display Total Cell Only OFF

Dilution Calculator Options

Current Concentration Default Value — Total Cells ON — Live Cells OFF — Dead Cells OFF

Printer Option

Print — Print Protocol ON — Thermal ON

Chapter 4 – Fluorescence Cell Counting

4.1 Sample Preparation

4.1.1 Prepare the following materials to count cells.

- Cell sample
- PhotonSlide™ (Cat # L12005) or LUNA™ Reusable Slide (Cat # L12008)
- Acridine Orange/Propidium Iodide Stain (Cat # F23001)

4.1.2 If desired, insert a USB drive to save data and results.

4.1.3 Transfer 2 μl of the Acridine Orange/Propidium Iodide Stain to a new 1.5 ml microfuge tube.

4.1.4. Add 18 μl of the cell sample to the tube and mix by pipetting up and down or flicking the bottom of the tube while holding with the other hand.

4.2 Loading Samples into Slides

4.2.1 Holding the edge of the slide, load 10 μl of the mixed cell sample into the inlet of one chamber of the counting slide. For easy and accurate loading, tilt the pipette around 45-60 degrees as shown below.

<Sample Loading>

<Slide Inserting>

4.2.2 The remaining 10 µl of the sample can be used for another duplicate counting to get average the counting results.

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

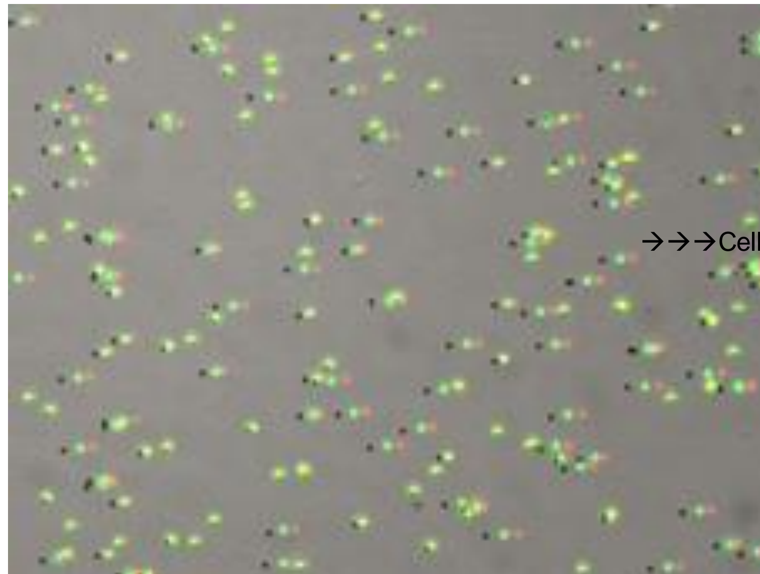
4.3 Counting Cells

4.3.1 Wait for 10 sec-1 min (depending on the sample condition) in order for the cells to settle down. This step is very important.

Note: Skipping this step may lead to poor counting accuracy because moving cells can generate poorly aligned cell images from the bright field, green and red channel, respectively. Optimal waiting time should be determined empirically. If cells are still moving during the preview step (step 4.3.4), a longer waiting time is required. Make sure that cells do not move by observing them in the preview screen.

Note: In case that the cells do not settle down, the captured images from bright field and green/red fluorescence do not align exactly, as shown below.

<Bright Field, Green, & Red Images of Unsettled Cells>



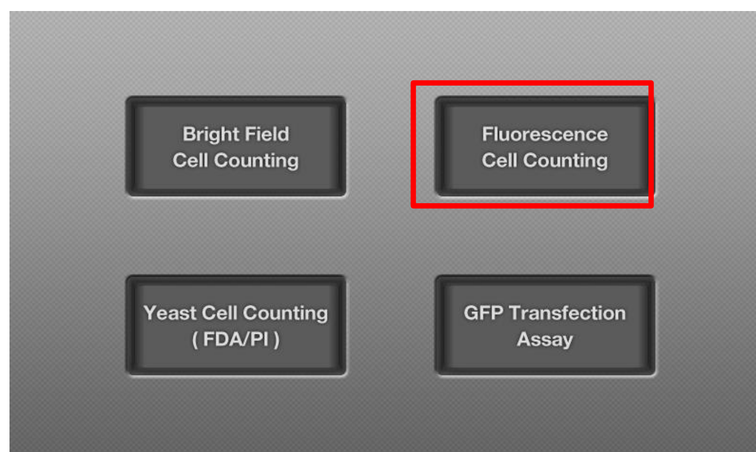
4.3.2 Insert the loaded slide into the slide port of the instrument, ensuring that the loaded chamber is inserted first into the slide port. The instrument analyzes only the first inserted chamber. Gently insert the counting slide to the end.

Note: After inserting the slide, LUNA-FL™ only reads the first chamber. To read the second chamber, it must be taken out, rotated, and inserted again.

Note: Make sure that the counting slide is not inserted upside-down. This may lead to sample spilling and could severely damage the counter.

4.3.3 Select the “Fluorescence Cell Counting” button on the home screen.

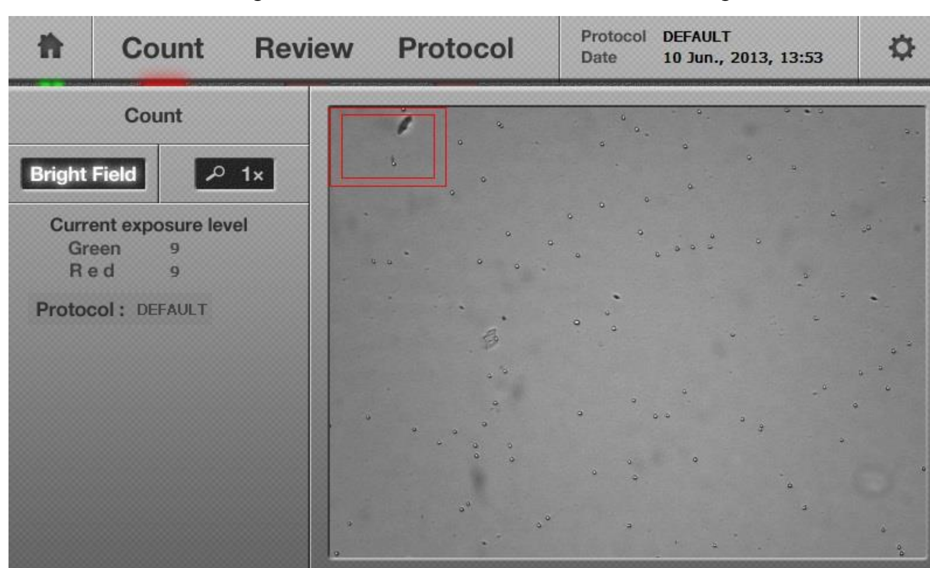
<Home Screen>



Note: After this step, the real image of the cell sample will be displayed on the screen. The cell image can be navigated by touching the screen and moving the finger or stylus pen on the image. Please note that the touch screen of the LUNA-FL™ Automated Fluorescence Cell Counter is a resistive touch screen, which needs a bit of pressure in order to get a response.

4.3.4 The “Preview” screen in the bright field mode will appear as shown in the image below. Make sure that users have the best focus. When necessary, adjust the focus with the focus knob on the right side of the instrument.

<Bright Field Mode in Fluorescence Counting>



Note: Make sure that the bright field mode is selected to adjust the focus. The right focus is very important for accurate counting.

<Adjusting the Focus>

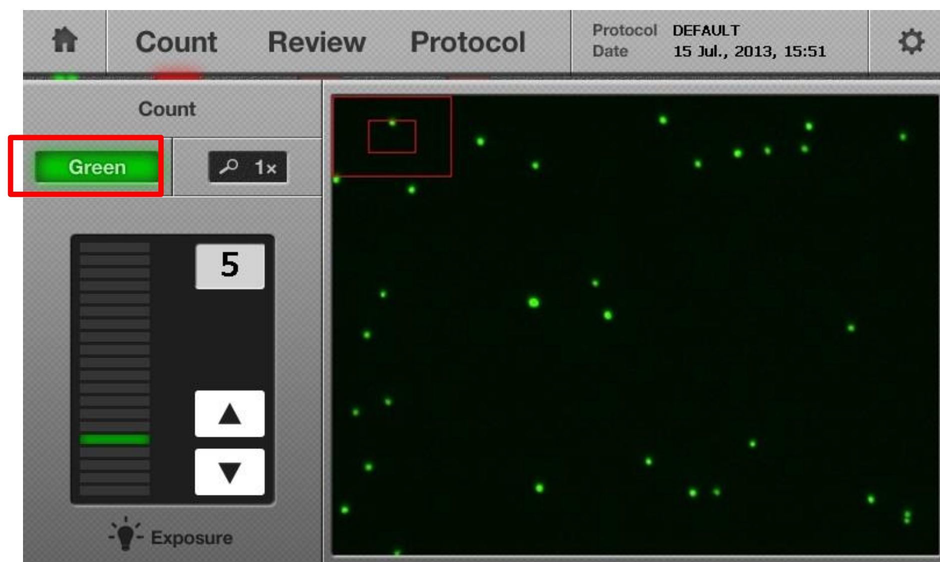
Note: To get the best focus, the image can be magnified by using the “Zoom-in” button located under the “Count” button. Initially, the “Zoom-in” button is set to a 1x image. When pressed once, a 2x image appears. This 2x image may be the best zoom-in status for focusing. When pressed again, a 4x image will be shown. Pressing the button again takes the screen back to a 1x image.

Note: Check if all cells in the preview screen are immobile. If some cells are still moving, wait for all cells in the preview screen to stop moving.

4.3.5 Press “BF/GF/RF selection button” once to preview the green fluorescence (GF) image. Increase the GF intensity value if the preview image is too dim so that only some cells are visualized. Decrease the GF intensity value if the preview image is too bright so that background fluorescence starts to come up. The optimal GF intensity value should be determined empirically. The factory set value for mammalian cells is 5.

Note: During the preview step, if cells are irradiated for a long period of time, their fluorescent intensities will quickly decrease. This bleaching phenomenon is accelerated when a higher level of fluorescence is used. Always try to use the shortest preview time and the lowest fluorescence level when the GF or RF preview is selected.

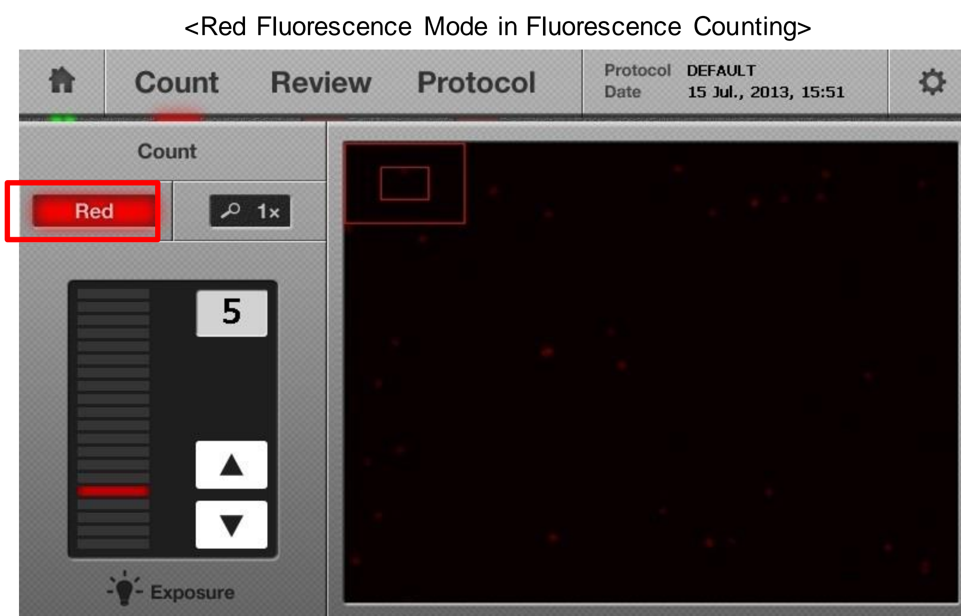
<Green Fluorescence Mode in Fluorescence Counting>



4.3.6 Press “BF/GF/RF selection button” one more time to preview the red fluorescence (RF) image. Adjust RF intensity value as needed. The factory set value for mammalian cells is 5.

Note: During the preview step, if cells are irradiated for a long period of time, their fluorescent intensities will quickly decrease. This bleaching phenomenon is accelerated when a higher level of fluorescence is used. Always try to use the shortest preview time and the lowest fluorescence level when the GF or RF preview is selected.

<Red Fluorescence Mode in Fluorescence Counting>

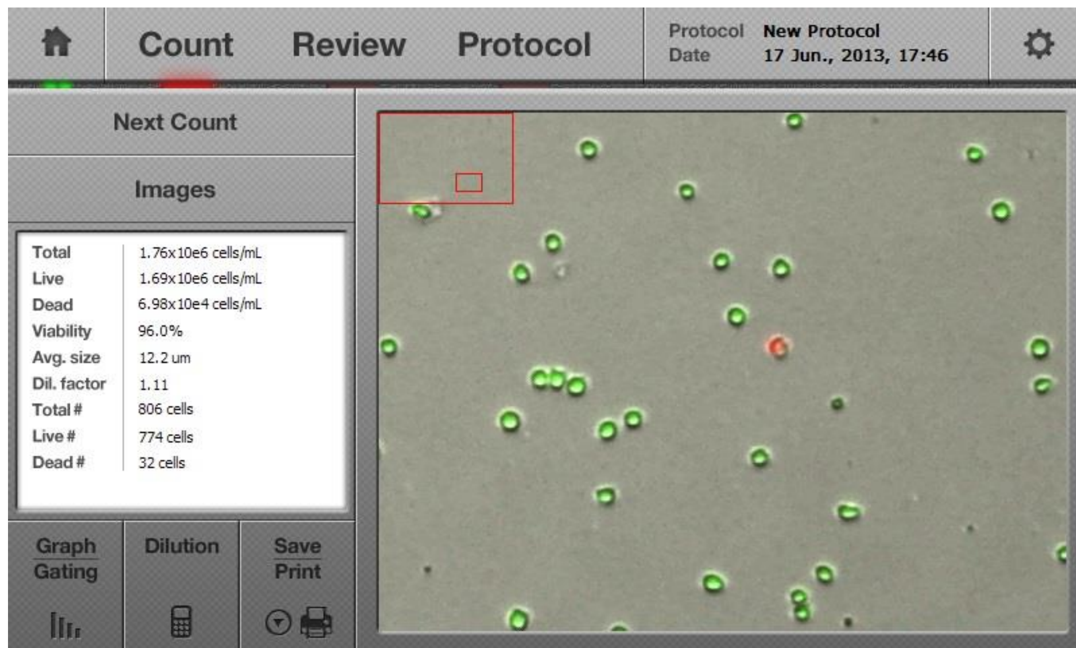


4.3.7 Press the “Count” button.

4.3.8 Within approximately 30 seconds (depending on cell concentration), the image of the cell sample and data/results (Total, Live, Dead, Viability, avg. size, total number, live number and dead number) will be displayed on the screen.

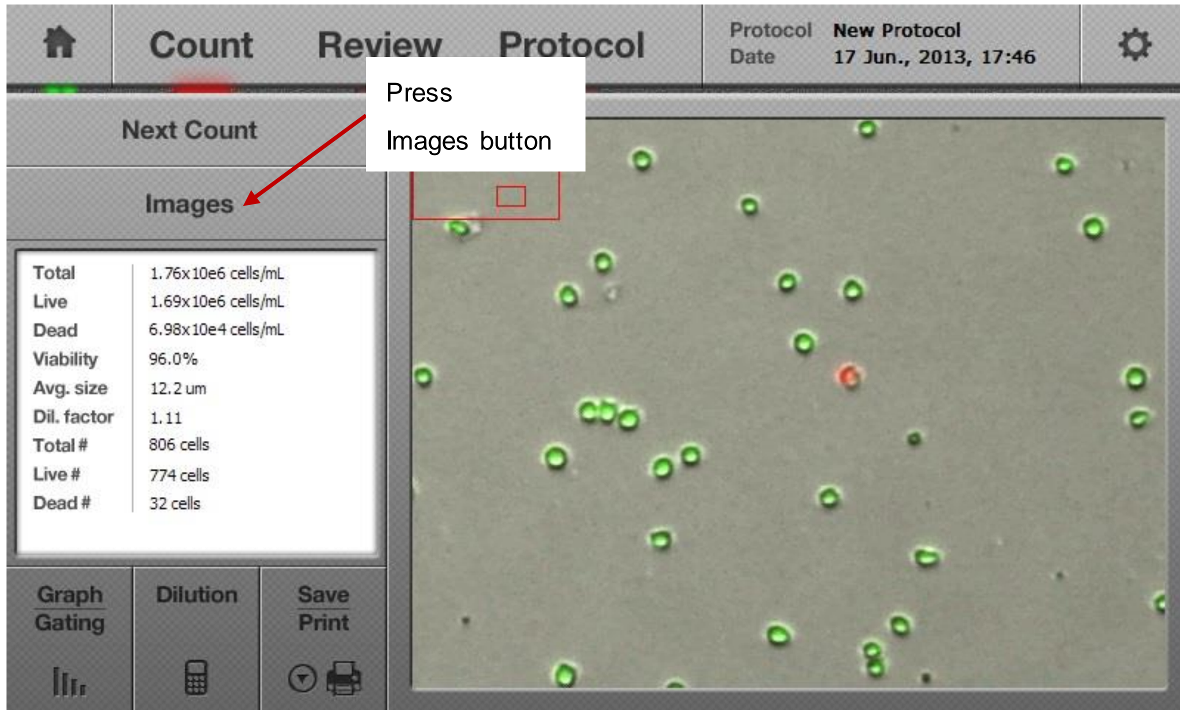
Note: The LUNA-FL™ take 3 images from the bright field, green, and red channel sequentially.

<Fluorescence Cell Counting Results>



4.4 Using the “Tag” Function

4.4.1 To verify counting results after performing the counting function, press “Images” and “Tag” buttons as shown below.



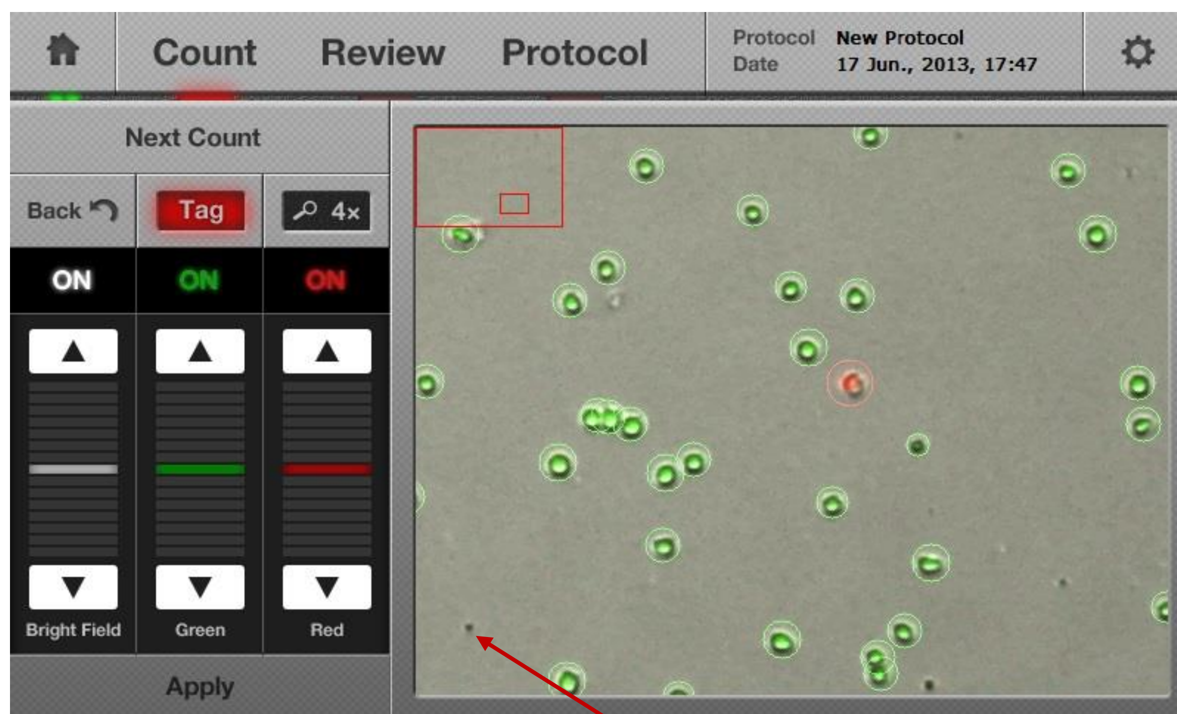
<Tagged Cell Image>

Green circle – live cell Red circle – dead cell

Note: This "Tag" function is one of the distinct tools of the LUNA-FL™ Automated Fluorescence Cell Counter because it helps users review and determine the accuracy of the counting on-site without depending on a computer.

4.4.2 When touching the "Tag" button, the image on the screen will show objects (mostly cells) surrounded by green or red circles. The green circles indicate live cells and the red circles indicate dead cells.

Note: In the "Fluorescence Cell Counting" mode, non-cellular debris contained a cell culture medium can be easily identified and automatically excluded from cell counting because it does not have any nucleic acids.



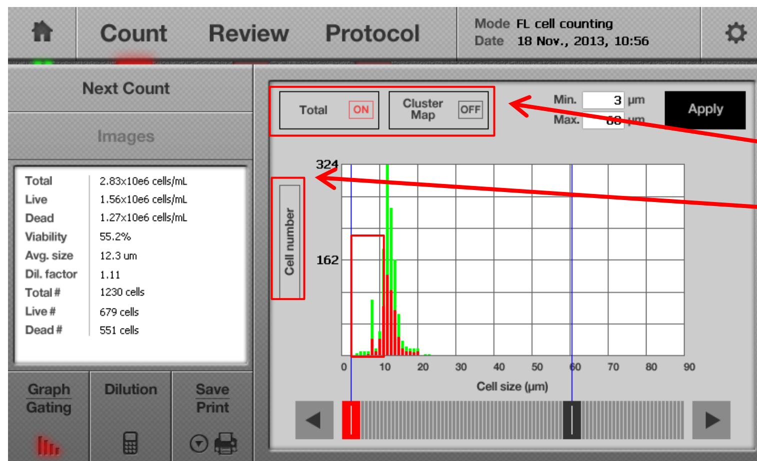
Non-cellular debris

4.4.3 After reviewing the accuracy of the image analysis, the "Tag" button can be pressed again to remove the green and red circles.

4.5 Cell Size and Number Distribution

4.5.1 To obtain more data on the distribution of cell size and number in graphical representation, press the "Graph/Gating" button on the lower left side of the screen. A histogram will appear with more details on the size and number of the cell sample as shown below. Live cells are displayed as a green bar and dead cells are displayed as a red bar.

<Graphical Representation of Cell Size Distribution>



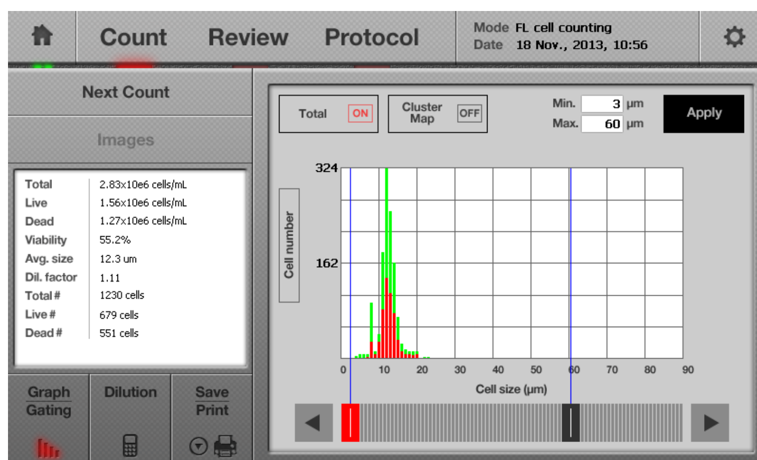
Functional buttons in the menu "Count → Graph/Gating"

1) "Total, Live or Dead" button
2) "Cluster Map" button
3) "Cell number or /ml" button

* The above buttons can be used in combination and the details are described in next pages.

Note: When the sizes of the cells are out of the range, they are displayed in grey color (not shown here).

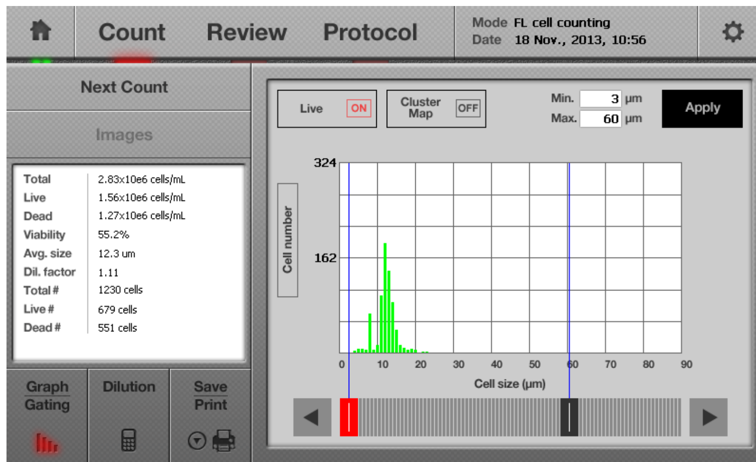
<Combination: Total and Concentration>



Combination of "Total" and "Cell number" shows the distribution of cell size vs. cell number for total cells.

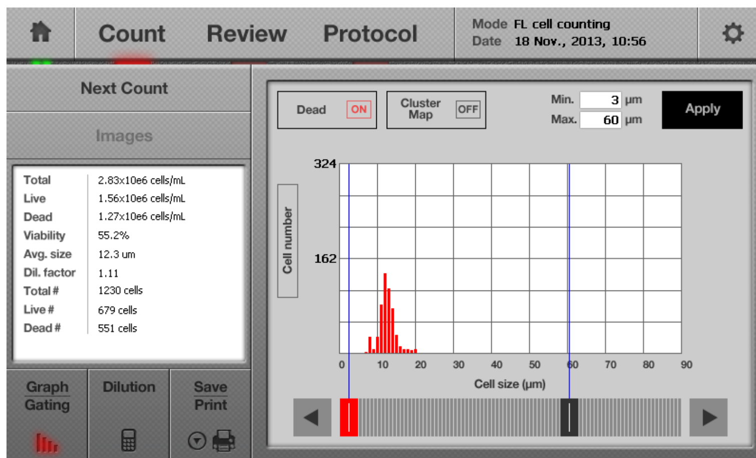
Note: When the "Total" button is pressed, it toggles to the "Live" button, which shows only live cells as shown below. When the button is pressed again, it toggles to the "Dead" button to show only dead cells as in the figure below.

<Combination: Live Cells and Cell Number>



The combination of "Live" and "Cell number" shows the distribution of cell size vs. cell number for only live cells.

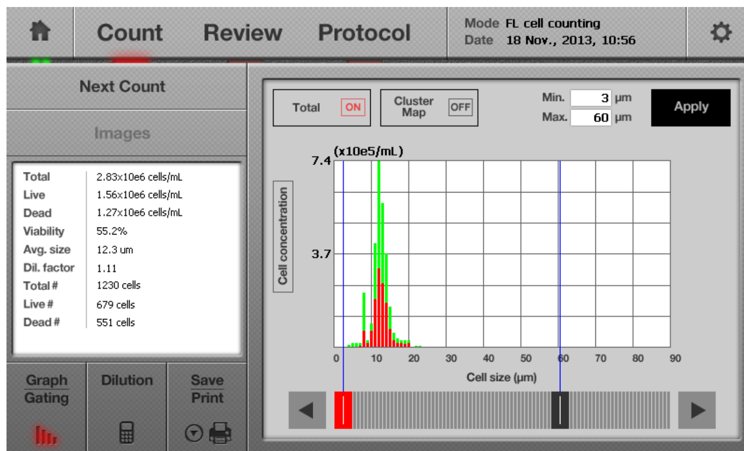
<Combination: Dead Cells and Cell Number>



The combination of "Dead" and "Cell number" shows the distribution of cell size vs. cell number for only dead cells.

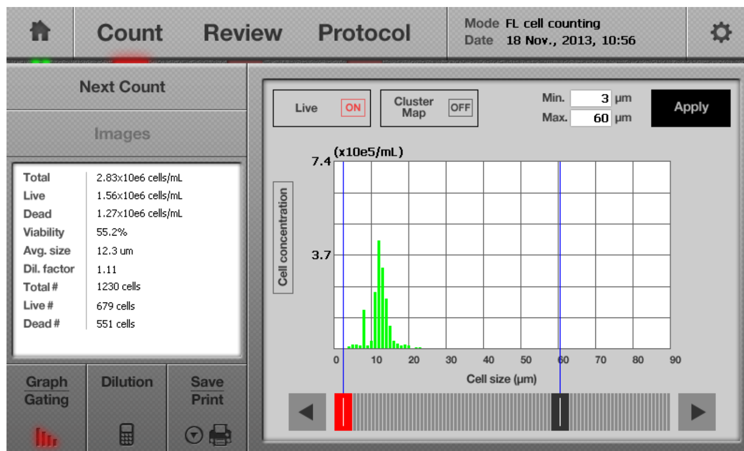
Note: When the "Cell Number" button is pressed, it toggles to the "/ml" button which shows the cell concentration (i.e., the Y axis of the histogram). When pressed again, it toggles to the "Cell Number" button.

<Combination: Total and Concentration>



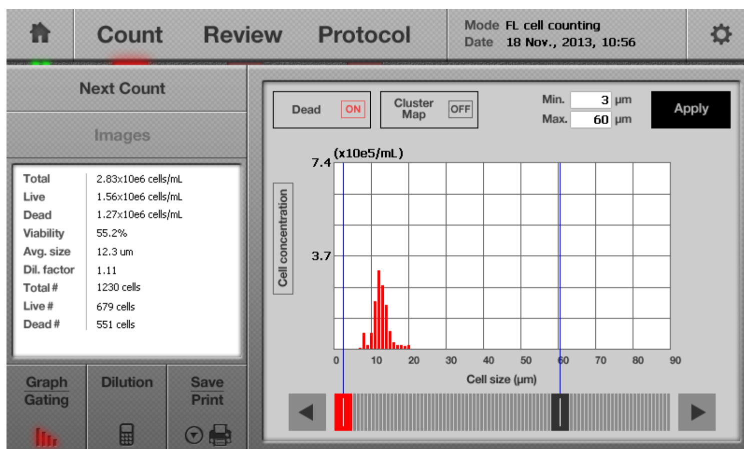
The combination of “Total” vs. “Cell concentration” shows the distribution of cell size vs. cell concentration for total cells.

<Combination: Live and Concentration>



The combination of “Live” vs. “Cell concentration” shows the distribution of cell size vs. cell concentration for only live cells.

<Combination: Dead and Concentration>

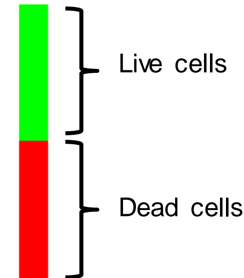


The combination of “Dead” vs. “Cell concentration” shows the distribution of cell size vs. cell concentration for dead cells.

4.5.1.1 Graphical representation of live and dead cells

When the "Total" button icon, the histogram is expressed as a "Stacked Column Chart."

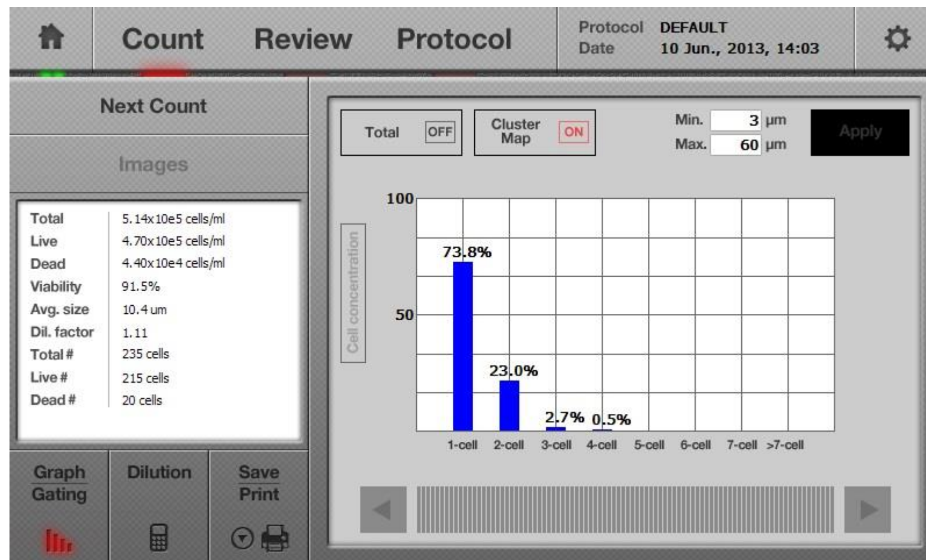
<Graphical representation of live and dead cells>



4.5.2 "Cluster Map" button

4.5.2.1 When the "Cluster Map" button is pressed, the percentage distribution of the clustered cells are displayed. "1-cell" means single cell and "2-cell" means 2 clustered cells as a unit. When the "Cluster Map" button is on, the "Total" and "Cell Number" buttons become inactivated.

<Cluster Map>

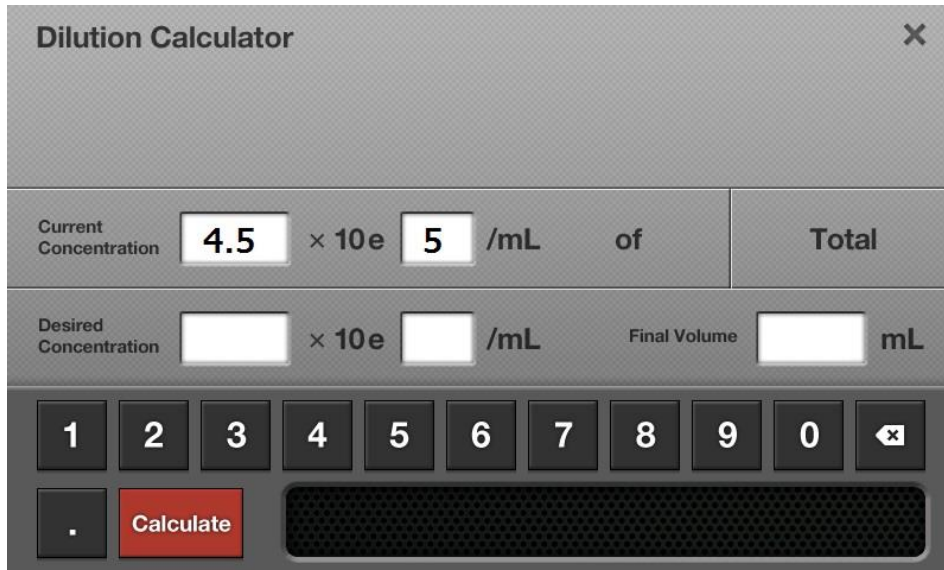


4.5.2.2 Press the "Graph/Gating" button again to go back to the previous screen.

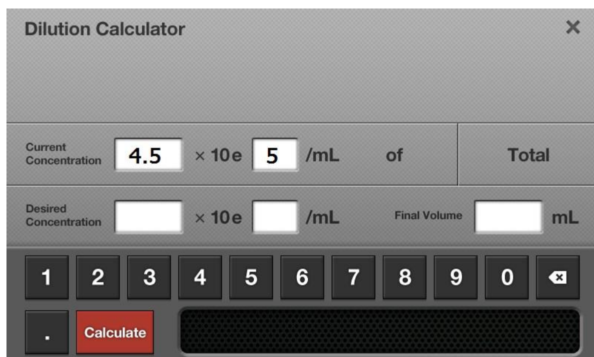
4.6 Using the Dilution Calculator

4.6.1 The LUNA-FL™ Automated Fluorescence Cell Counter provides a built-in dilution calculator that helps easily calculate adjustments to obtain a desired concentration.

<A Built-in Dilution Calculator>



4.6.2 The dilution calculator initially shows the current concentration. Put the appropriate numbers into the blanks of the “Desired Concentration” and “Final Volume” that you want to obtain.



Users can choose one of “Total”, “Live” or “Dead” cells for calculating their dilution.
By clicking this button, the “Current Concentration” displays the concentration of the total, live or dead cells.
Before calculating the dilution factor, please check this option to match your requirements.

Users can also calculate their custom dilution.

4.6.3 Press the “Calculate” button and then a dilution instruction will appear in the message box.

4.7 Saving the Image and Generating a Report of the Current Count

4.7.1 To save data and/or generate a report, insert a USB drive into the USB port on the left side of the instrument.

4.7.2 Press the "Save" button located on the lower left part of the screen and then a new "Save" window will pop up on the screen as shown below.

<Save and Report Screen>

Note: This "Save" window contains three check boxes as follows:

- Analyzed Image: Check this box to save an analyzed image. Live cells are tagged in green and dead cells in red for further analysis.
- Raw Image: This box must always be checked in order to save the image in TIF format. The saved image can be re-analyzed on the instrument later if necessary. Remember to save the "Raw Image" for getting technical support from distributor or manufacturer.
- Report: Check this box to generate a printed report as a PDF. The report contains all the information of the cell counting session. This report can be read and printed by a personal computer (PC). Please un-check if a report is not necessary.

Note: Remember to save and send the "Raw Image" for getting technical support from distributor or manufacturer.

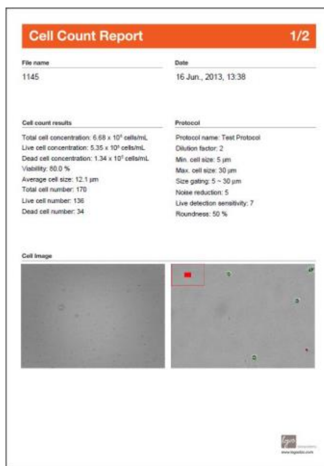
4.7.3 Enter the file name using the keyboard buttons on the screen. The date and time can be added by pressing the “Date/Time” button.

Note: Make sure that the cursor is located in the file name box before entering the file name.

4.7.4 Once the file name is verified, press the “Enter” button to save the image and/or report into the USB drive. Now, the saved files can be opened after transferring them via USB drive to a PC with the appropriate software.

Note: With the LUNA-FL™ software, additional graphical representations of the data and results are provided in the PDF report, as shown below.

<Two page PDF report generated by LUNA-FL>



Cell Count Report 1/2

File name: 1145

Date: 16 Jun., 2013, 13:38

Cell count results

Total cell concentration: 6.68 x 10^5 cells/mL
Live cell concentration: 5.35 x 10^5 cells/mL
Dead cell concentration: 1.34 x 10^5 cells/mL
Viability: 80.0 %
Average cell size: 12.1 µm
Total cell number: 170
Live cell number: 136
Dead cell number: 34

Protocol

Protocol name: Test Protocol
Dilution factor: 2
Min. cell size: 5 µm
Max. cell size: 30 µm
Size gating: 5 ~ 30 µm
Noise reduction: 5
Live detection sensitivity: 7
Roundness: 50 %

Cell image

<1st page - Data and Results>
Cell counting data
Protocol used
Cell images (tagged and zoomed)

<2nd page - Analytical Histograms>
By cell number (Total/Live/Dead)
By cell concentration (Total/Live/Dead)
By cell clustering

4.8 Printing the Counting Results

Please see Section 3.8 to print the counting results.

Chapter 5 – GFP Transfection Assay

5.1 Sample Preparation

5.1.1 Perform a GFP transfection experiment with the appropriate negative controls, e.g., non-transfected control or mock-transfected control without plasmid

5.1.2 Harvest the cell samples approximately 24 - 96 hours after the transfection.

5.1.3 Count the number of cells in the sample. The optimal cell concentration for cytometry is generally between 2 x 10^6 – 5 x 10^6 cells/ml. If the initial concentration is too high, add some cell culture medium to dilute. If the initial concentration is too low, centrifuge the cell suspension and resuspend the cells in a smaller volume to raise the cell concentration.

5.1.4 Turn on the LUNA-FL™ instrument and prepare the following items in order to measure GFP transfection efficiency.

- Cell sample
- PhotonSlide™ (Cat No. L12005) or LUNA™ Reusable Slide (Cat # L12008)
- A USB drive to save data and results, if desired.

Note: The reagents and kits for the GFP transfection assay are not included in the LUNA-FL™ package. They must be purchased separately from other suppliers.

5.2 Loading Samples into Slides

5.2.1 Holding the edge of the slide, load 10 µl of the negative control on one side and the GFP-transfected sample on the other side of the counting slide. For easy and accurate loading, tilt the pipette around 45-60 degrees as shown below.

<Sample Loading>

<Slide Inserting>

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

5.3 Measuring GFP Transfection Efficiency

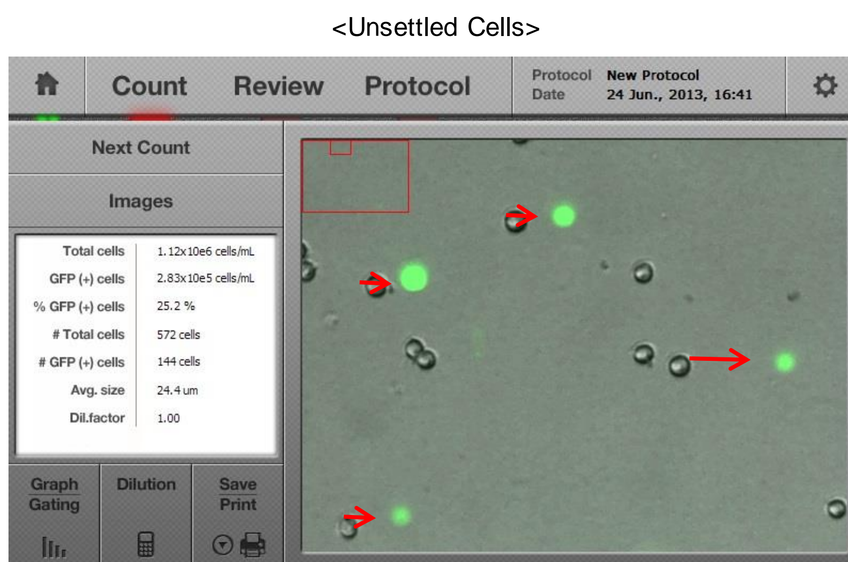
5.3.1. Optimization of the measuring conditions (Focus and Exposure level)

5.3.1.1 Wait for 10 sec - 1 min in order for the cells to stabilize.

Note: This step is important. Skipping the waiting step will result in poor accuracy. Optimal waiting time should be determined empirically. If cells are still moving during the preview step, a longer waiting time is required.

Note: In case the cells do not stabilize, the captured images from bright field and green fluorescence do not align exactly, as shown below.

<Unsettled Cells>



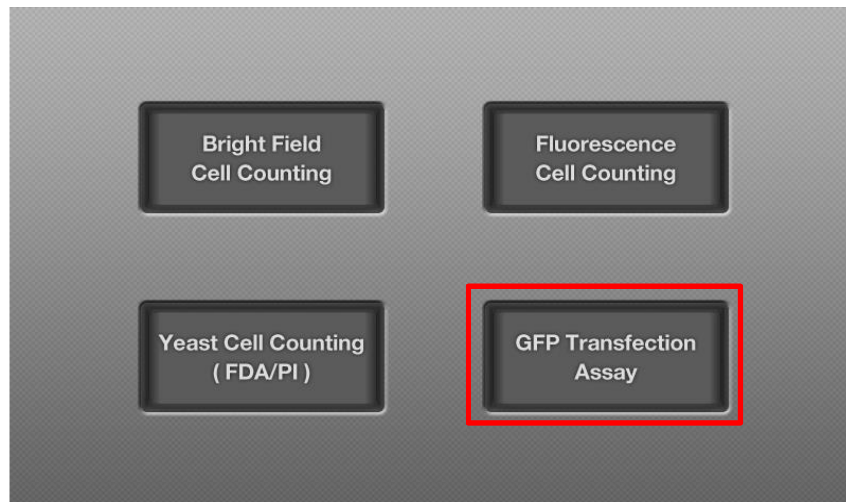
5.3.1.2 Insert the slide containing the GFP-transfected cells into the slide port of the instrument, ensuring that the loaded chamber is inserted first into the slide port. The instrument analyzes only the first inserted chamber upon counting. Gently insert the counting slide to the end.

Note: After inserting the slide, LUNA-FL™ only reads the first chamber. To read the second chamber, it must be taken out, rotated, and inserted again.

Note: Make sure that the counting slide is not inserted upside-down. This may lead to sample spilling and could severely damage the counter.

5.3.1.3 Select the “GFP Transfection Efficiency” button on the home screen.

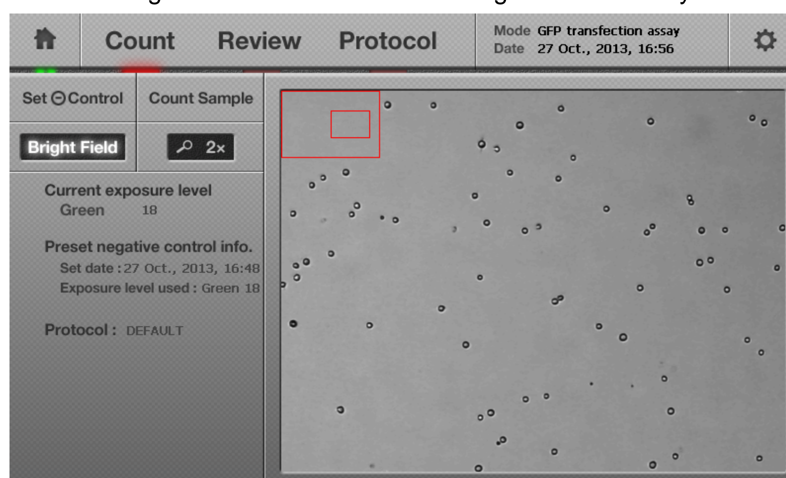
<Home Screen>



Note: After this step, the real image of the cell sample will be displayed on the screen (Preview status). The cell image can be navigated by touching the screen and moving a finger or stylus pen on the image. Please note that the touch screen of the LUNA-FL™ Automated Fluorescence Cell Counter is a resistive touch screen type, which needs a bit of pressure in order to get a response.

5.3.1.4 The “Preview” screen in the bright field mode will appear as shown in the image below. Make sure that the best focus is being used. When necessary, adjust the focus with the focus knob on the right side of the instrument.

<Bright Field Preview in Measuring GFP Efficiency>



Note: For the best results, use the bright field mode (2x or 4x) to adjust the focus.

<Adjusting the Focus>

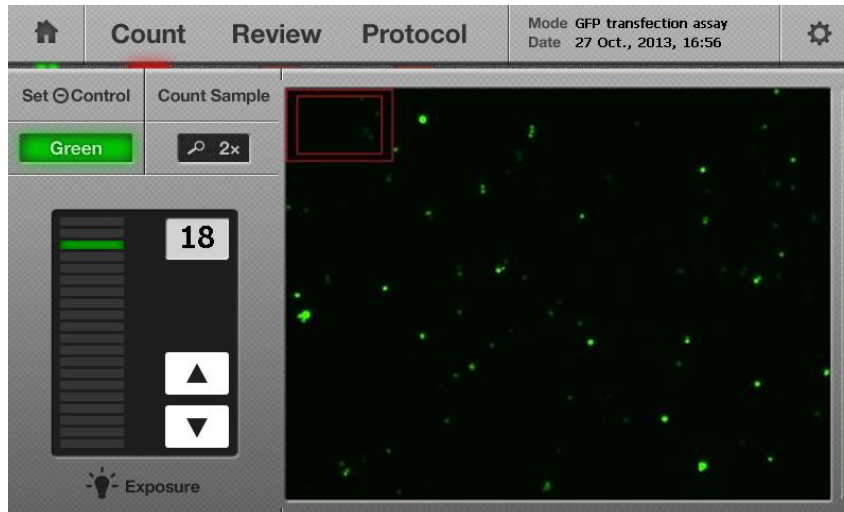
Note: To get the best focus, the image can be magnified by using the "Zoom-in" button located under the "Count Sample" button. Initially, the "Zoom-in" button is set to a 1x image. When pressed once, a 2x image appears. This 2X image may be the best zoom-in status for focusing. When pressed again, a 4x image is shown. Pressing the button again takes the screen back to a 1x image.

Note: Check that all cells in the preview screen are immobile. If some cells are still moving, wait for all cells in the preview screen to stop moving.

5.3.1.5 Press the "BF/GF selection" button once to preview the green fluorescence (GF) image. Increase the Green exposure level if the preview image is too dim so that only some cells are visualized. Decrease the Green exposure level if the preview image is too bright so that background fluorescence starts to interfere. Optimal exposure value should be determined empirically. The factory set value for mammalian cells is 18.

Note: During the preview step, if cells are irradiated for a long period of time, their fluorescent intensities will quickly decrease. This bleaching phenomenon is accelerated when a higher level of fluorescence is used. Always try to use the shortest preview time and the lowest fluorescence level when the GF preview is selected.

<Green Fluorescence Preview in Measuring GFP Transfection Efficiency >



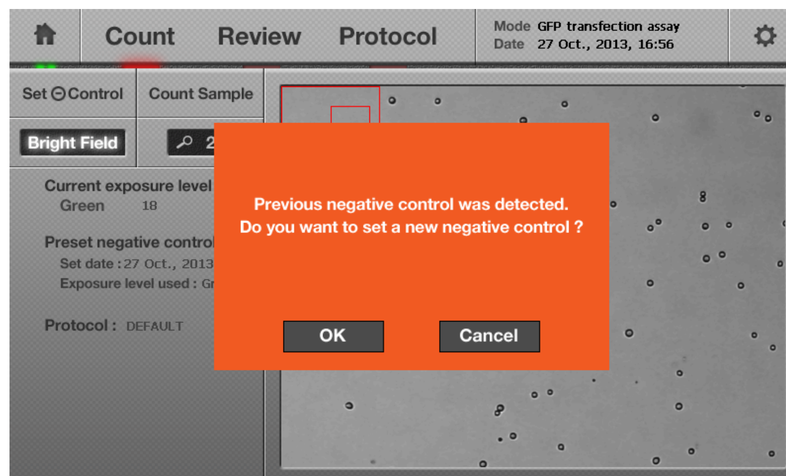
Note: The exposure level of the green fluorescence must be started from 18 and then, depending on the signal strength, it can be adjusted.

5.3.2 Setting the negative control

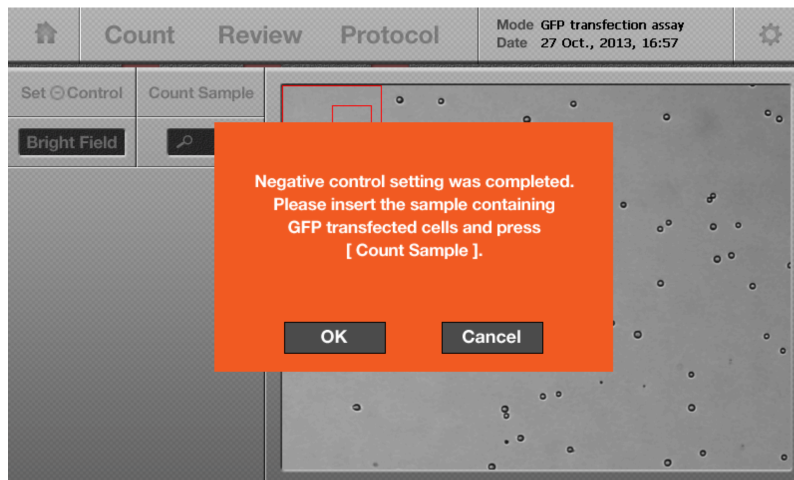
5.3.2.1 Remove the slide containing the transfected sample and insert a slide containing **the negative control**.

5.3.2.2 Press the "Set (-) control" button.

Note: A pop up window (as shown below) might show up to warn the presence of the previous negative control. If you pressed the "Set (-) control" button by mistake, press the "Cancel" button to go back. Otherwise, press the "OK" button to proceed.



5.3.2.3 Within approximately 30 seconds (depending on cell concentration), the analysis of the negative control will be done. Press the "OK" button.



5.3.3 Counting the GFP transfected sample

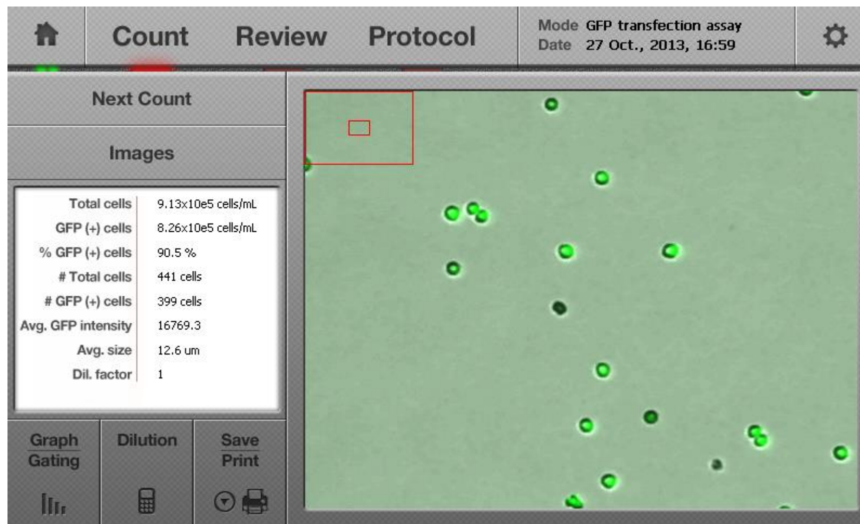
5.3.3.1 Insert a slide containing **the GFP-transfected sample** again and press the "Count Sample" button.

5.3.3.2 Within approximately 30 seconds (depending on cell concentration), the image of the cell sample and data/results (Total concentration, GFP (+) concentration, GFP (+) percentage, Total cell number, GFP (+) number, Avg. size, Dil. Factor) will be displayed on the screen.

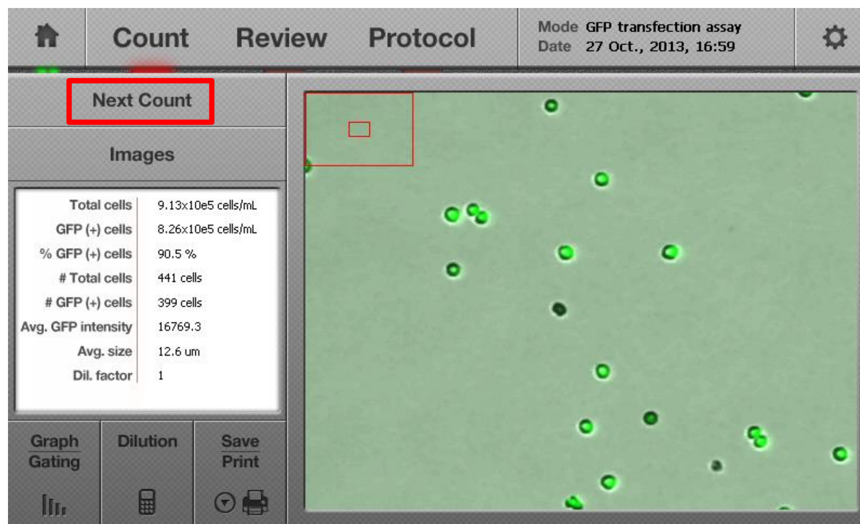
Note: Use the same exposure level either to the negative control and the sample.

Note: The GFP transfection data on the results screen is calculated with the automated gating function. For more accurate results, press the "Graph" button and perform a new gating. Refer to Section 4.5 for more detailed information about the user-defined gating.

<Result Screen>



5.3.3.3 If you have multiple GFP transfected samples and want to measure the efficiency of the next sample, insert the new slide and press the “Next Count” button.

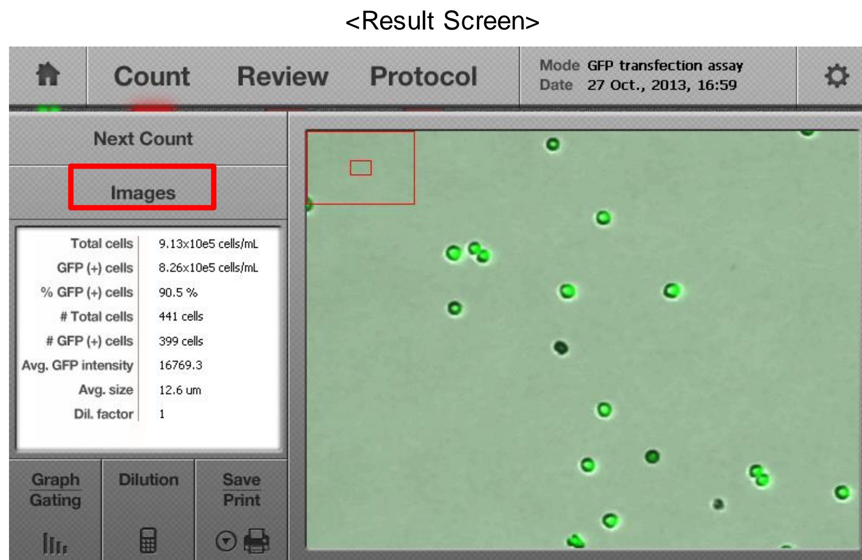


5.3.3.4 Press the “Count Sample” button as needed.

5.4 Using the “Tag” Function

5.4.1 To verify the results after performing the “measuring GFP transfection efficiency” function, press the “Images” and “Tag” buttons as shown below.

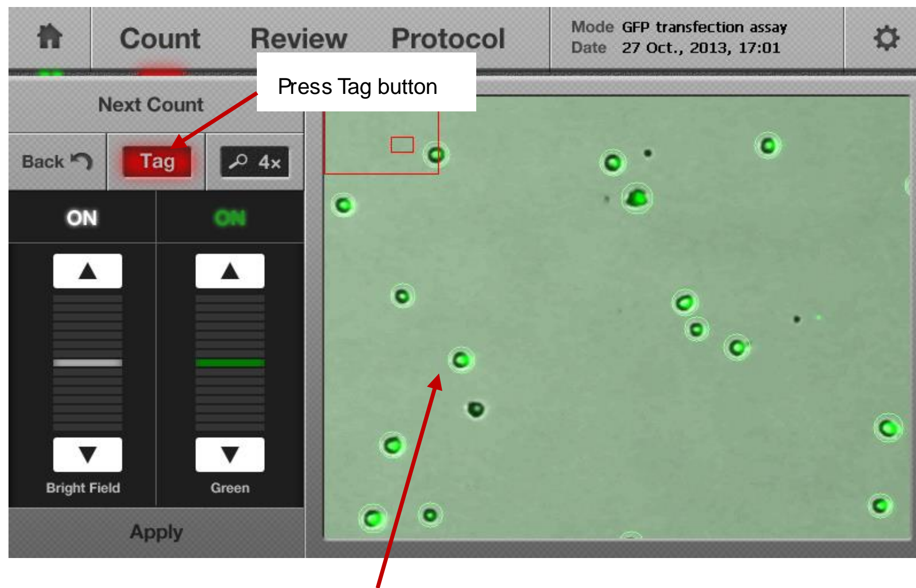
<Result Screen>



Note: This “Tag” function is one of the distinct tools of the LUNA-FL™ Automated Fluorescence Cell Counter because it helps users review and determine the accuracy of measuring GFP transfection efficiency on-site without depending on a separate computer.

5.4.2 When touching the “Tag” button, the image on the screen will show objects surrounded by green circles. The green circles indicate GFP-transfected cells.

<Tagged Cell Image>

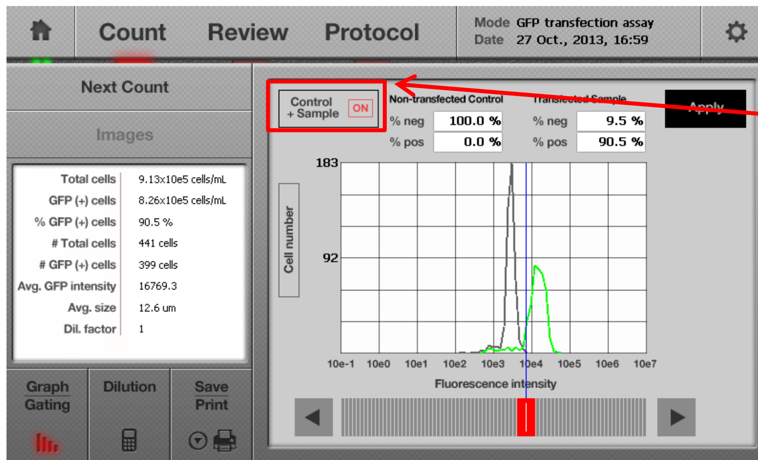


5.4.3 After reviewing the accuracy of the image analysis, “Tag” button can be pressed again to remove the green circles.

5.5 GFP Intensity and Number Distribution

5.5.1 To obtain more data on the distribution of GFP intensity and number in graphical representation, press the “Graph/Gating” button on the lower left side of the screen. A histogram will appear with more details on the GFP intensity and number of the cell sample as shown below. GFP-transfected cells are displayed as a green line and negative control cells are displayed as a grey line.

<Graphical Representation of the Distribution of GFP Intensity >



Functional buttons in the menu

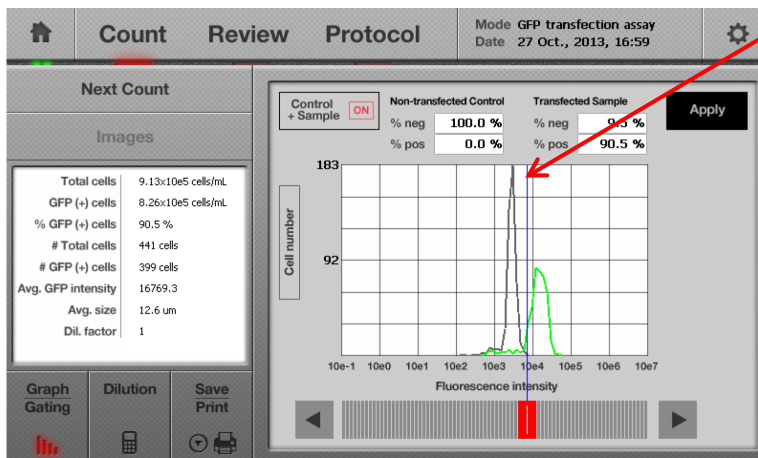
“Count → Graph/Gating”

1) “Control + Sample”

2) “Control” button

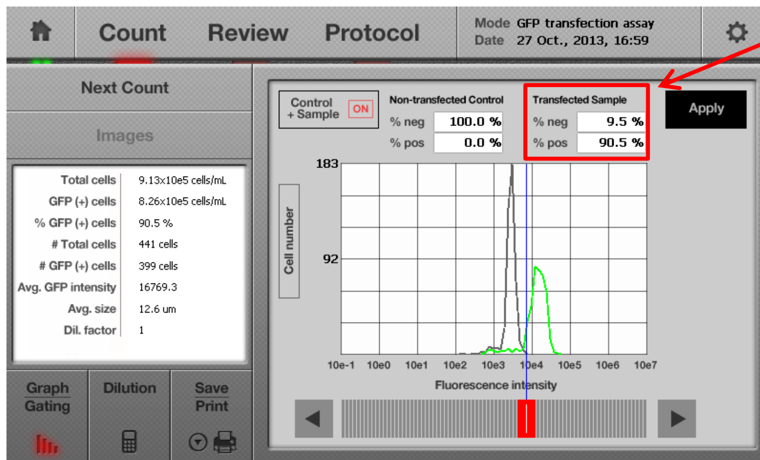
3) “Sample”

<GFP Intensity Threshold>



GFP Intensity Threshold is automatically determined by the LUNA-FL™ software.

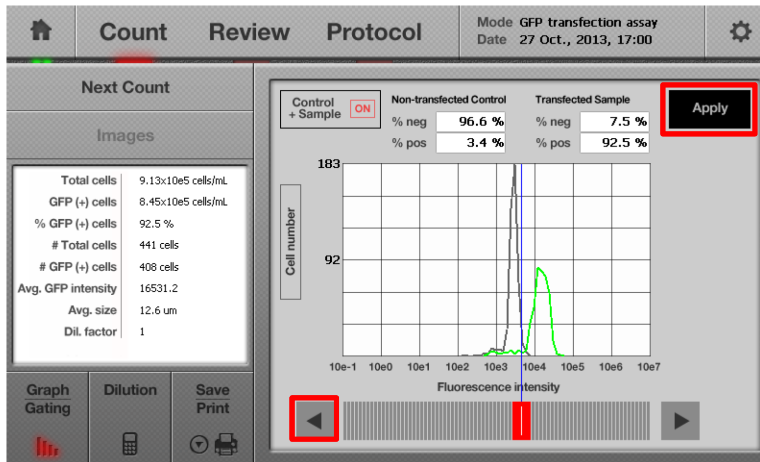
<Percentages of GFP (-) and GFP (+) cells>



Percentages of GFP (-) and GFP (+) cells of the sample are shown here.

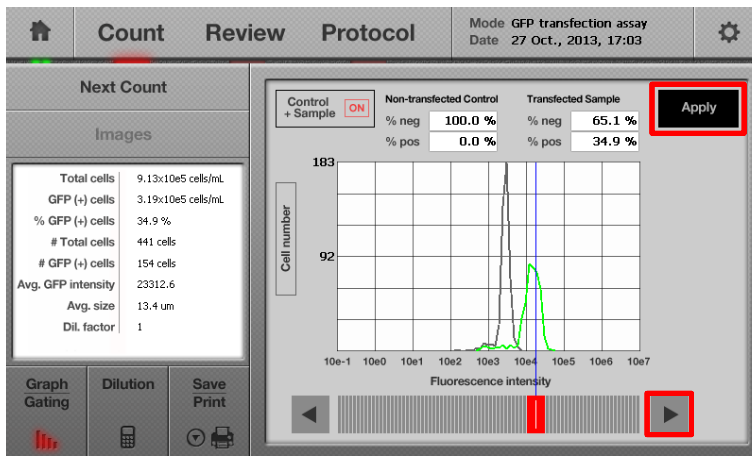
Note: The default threshold automatically set by the instrument is the smallest GFP intensity value, where 100% of negative control cells are classified as GFP (-) cells. In case the threshold drawn automatically is not satisfactory, users can re-assign the threshold as shown in the next figure to meet their specific needs.

<User-defined GFP Intensity Threshold (1)>



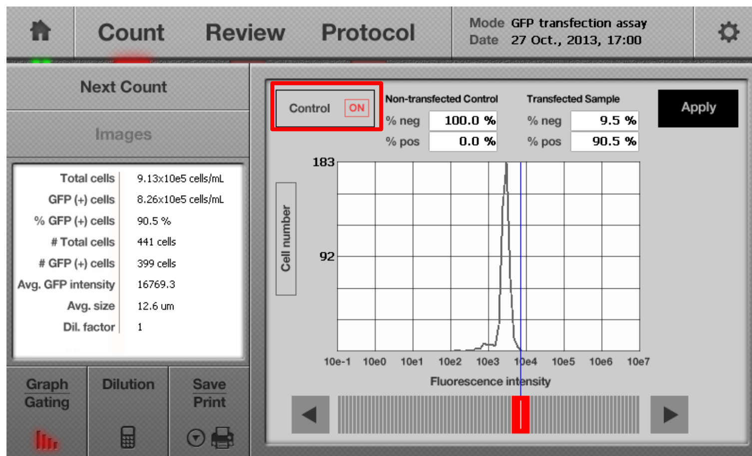
GFP Intensity Threshold can be decreased by users. Users can touch the left arrowhead icon located in the lower right corner. To update the percentage values of GFP (-) and GFP (+) cells, the “Apply” button should be pressed. Please note that the percentage values of GFP (-) and GFP (+) cells changed from the previous figure.

<User-defined GFP Intensity Threshold 2>



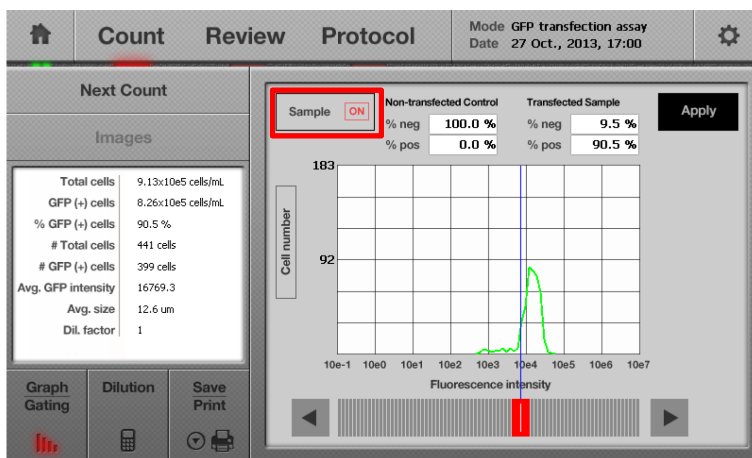
GFP Intensity Threshold can also be increased by users. Users can touch the right arrowhead icon located in the lower left corner. To update the percentage values of GFP (-) and GFP (+) cells, the "Apply" button should be pressed. Please note that the percentage values of GFP (-) and GFP (+) cells changed from the previous figures.

<GFP intensity of the control cells>



When the "Control + Sample" button is pressed once, the GFP intensities of the control cells display.

<GFP intensity of the GFP-transfected cells>



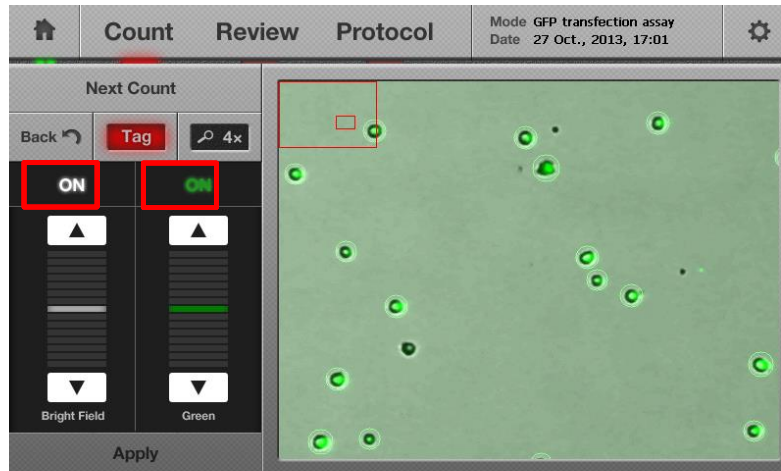
When the "Control" button is pressed again, the GFP intensities of the GFP-transfected cells display.

5.5.2 Press the "Graph/Gating" button again to go back to the previous screen.

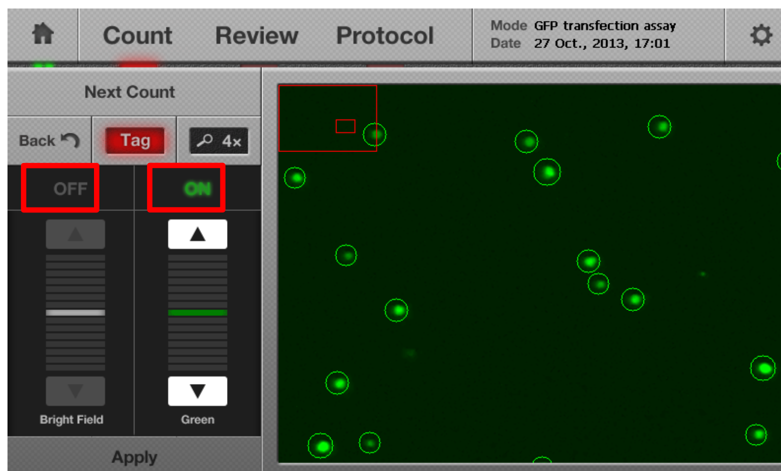
5.6 On/off of the Bright and Green Channel

5.6.1 Users can turn on or off of the bright and green channels each by clicking the “On/Off” button.

<Bright Field (on) and Green (on)>

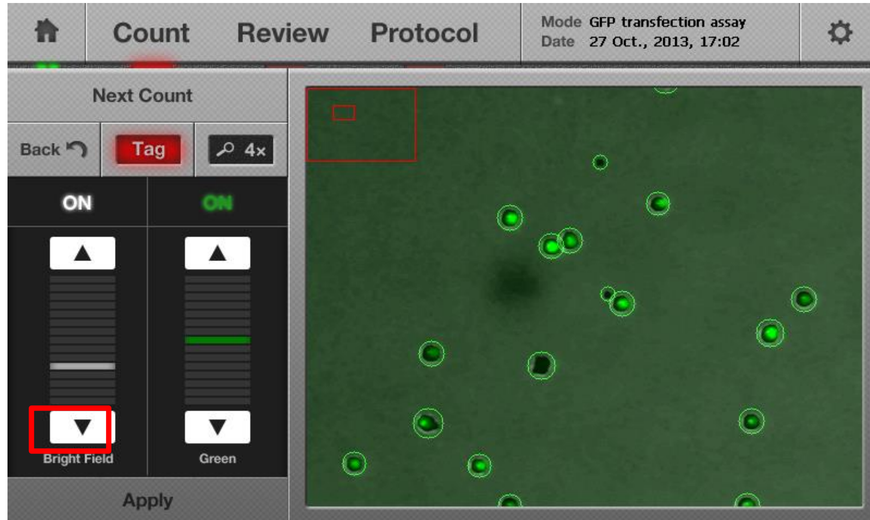


<Bright Field (off) and Green (on)>



5.6.2 Users can adjust the level of the bright and green channels each by clicking the lowering arrowhead icon.

<The level of Bright Field was lowered.>

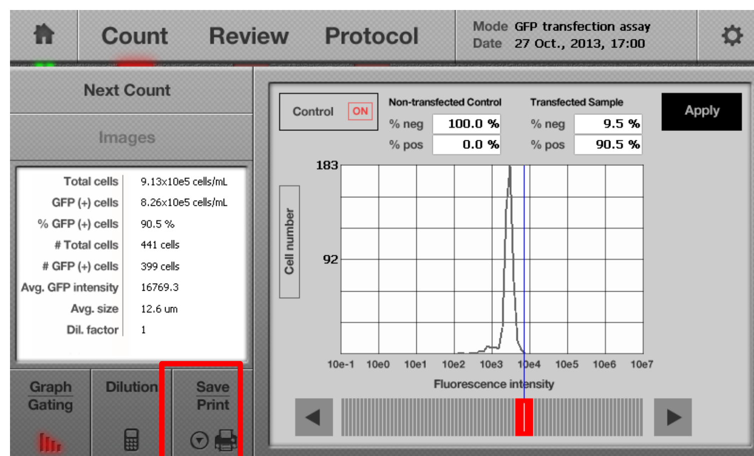


5.7 Saving the Image and Generating a Report of the Current Count

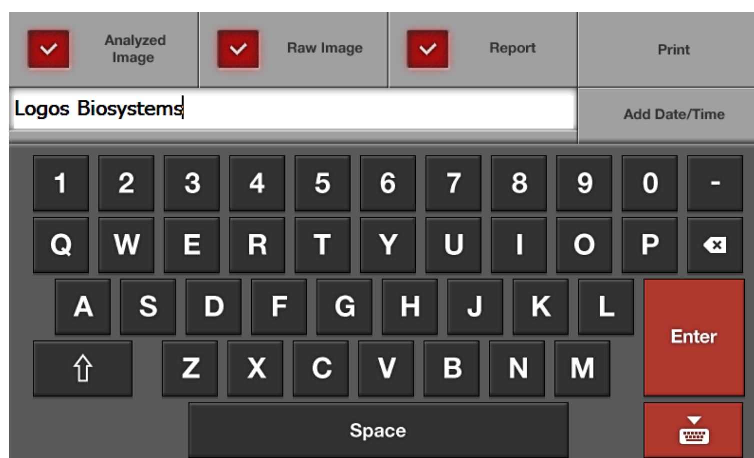
5.7.1 To save data and/or generate a report, insert a USB drive into the USB port on the left side of the instrument.

5.7.2 Press the “Save” button located on the lower left part of the screen and then a new “Save” window will pop up on the screen as shown below.

<Save/Print Button>



<Save and Report Screen>



Note: This "Save" window contains three check boxes as follows:

- Analyzed Image: Check this box to save an analyzed image. Live cells are tagged in green and dead cells in red for further analysis.
- Raw Image: This box must always be checked in order to save the image in TIF format. The saved image can be re-analyzed on the instrument later if necessary. Remember to save the "Raw Image" for getting technical support from distributor or manufacturer.
- Report: Check this box to generate a printed report as a PDF. The report contains all the information of the cell counting session. This report can be read and printed by a personal computer (PC). Please un-check if a report is not necessary.

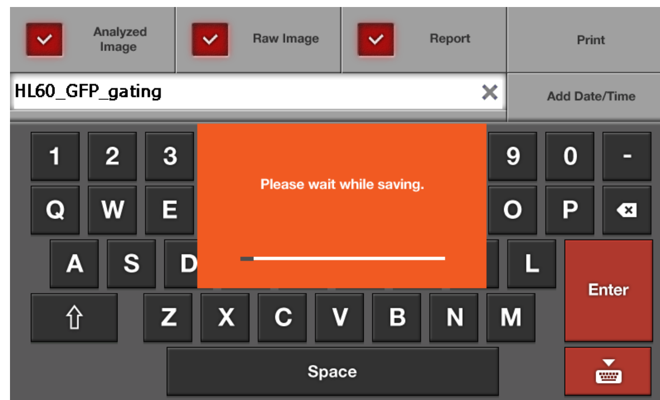
Note: Remember to save and send the "Raw Image" for getting technical support from distributor or manufacturer.

5.7.3 Enter the file name using the keyboard buttons on the screen. The date and time can be added by pressing the "Date/Time" button.

Note: Make sure that the cursor is located in the file name box before entering the file name.

5.7.4 Once the file name is verified, press the "Enter" button to save the image and/or report into the USB drive. Now, the saved files can be opened on a PC with the appropriate software after transferring them via USB drive.

<Images and Report Saving>



Note: With the LUNA-FL™ software, additional graphical representations of the data and results are provided in the PDF report, as shown below.

<Two page PDF report generated by LUNA-FL>

<1st page - Data and Results>	<2nd page – Fluorescence Intensity Histograms>
● Cell counting & GFP(+) data ● Protocol used ● Cell images (tagged and zoomed)	● Sample + Control ● Sample ● Control
Cell Count Report 1/2	Cell Count Report 2/2

5.8 Printing the Counting Results

Please see Section 3.8 to print the counting results.

Chapter 6 – Yeast Cell Counting

With a new reagent, Fluorescein Diacetate (FDA) and the software version 2.0 or higher, users of the LUNA-FL™ Automated Fluorescence Cell Counter can count yeast cells. For more information, please refer to the application note on the website (www.logosbio.com).

6.1 Sample Preparation

6.1.1 Prepare the following materials to count yeast cells.

- Yeast cells
- PhotonSlide™ (Cat # L12005) or LUNA™ Reusable Slide (Cat # L12008)
- Yeast Viability Kit 1 (Cat # F23202)

Note: The components of the Yeast Viability Kit 1 are as follows:

Item	Volume	Storage Condition
Fluorescein Diacetate (FDA)	500 µl	-20 ℃ (desiccated, dark)
Propidium Iodide (PI)	500 µl	4 ℃ (dark)
LUNA-FL™ Cell Dilution Buffer	20 ml	4 ℃ - Room Temperature
Fluorescence signal enhancer 1	500 µl	-20 ℃

- Shelf life: 16 months from manufacture date if stored appropriately.
- FDA is dissolved in DMSO. User precaution is required.
- Fluorescence signal enhancer 1 can be toxic if exposed to human skin. Gloves and lab coat are always required.
- Each item above can be purchased separately. Please contact your local distributors.

6.1.2 Before getting started, if necessary, users can check the sample conditions as follows:

6.1.2.1 Take out 10 µl of your yeast sample (wine or beer), load it onto the counting slide without adding any reagent, and check out the preview images on LUNA-FL™.

(Checkpoint #1) The BF image is too dark (especially, the wine)?
(Checkpoint #2) The FL image is already bright (auto-fluorescence)?
(Checkpoint #3) Too many yeast cells are present in the BF image?

6.1.2.2 If the answer is “yes” to any of the checkpoints above, the sample should be diluted with the LUNA-FL™ dilution buffer. The exact dilution ratio should be determined empirically. 1:10-1:100 dilution

is a good starting point. If needed, centrifuge the sample, remove the supernatant and resuspend the pellet with the yeast dilution buffer. After the dilution or washing step, proceed to the next step.

IMPORTANT

If your yeast cells were grown in the YPD medium, be sure to dilute the sample at least 1:100 with the Cell Dilution Buffer before counting. The component of the YPD medium has a strong esterase activity and inhibits the uptake of FDA into the yeast cells, leading to weak fluorescence signal.

6.1.2.3 If the answers are all no, just proceed to the next step.

6.1.3 Transfer 1 µl of FDA and 1 µl of PI to a new 1.5 ml microfuge tube.

Note: Whereas AO and PI measure the amount of nucleic acid of cells, FDA measures the activity of metabolic enzymes (Esterase) of cells. Whereas the amount nucleic acid does not vary greatly between different yeast strains, the activity of metabolic enzymes can vary in much higher degree between different yeast strains. Even the same strain of yeast can have different levels of enzymatic activity depending on their growth status. The variability of the signal strength of FDA is somewhat inevitable between different strains and growth status. In some strains, the difference becomes significant (for example, Brettanomyces) and in some strains, the difference is minimal (for example, Cerevisiae).

6.1.4. Add 18 µl of the sample to the tube and mix by pipetting up and down or flicking the bottom of the tube while holding with the other hand.

Note: Any solutions containing FDA and PI should be protected from strong light.

6.1.5 Incubate the mixed sample for 10 min.

Note: Due to the working principle of FDA, please make sure to incubate the mixed sample properly at room temperature.

6.2 Loading Samples into Slides

6.2.1 Holding the edge of the slide, load 10 µl of the mixed cell sample into the inlet of one chamber of the counting slide. For easy and accurate loading, tilt the pipette around 45-60 degrees as shown below.

<Sample Loading>

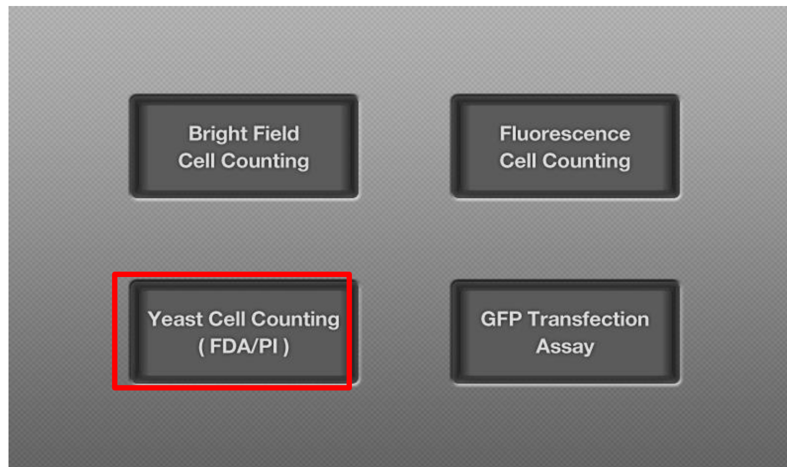
6.2.2 The remaining 10 µl of the sample can be used for another duplicate counting to get average the counting results.

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

6.3 Counting Yeasts

6.3.1 Turn on the instrument and select the “Yeast Cell Counting (FDA/PI)” button on the home screen.

<Home Screen>



6.3.2 Insert the loaded slide into the slide port of the instrument, ensuring that the loaded chamber is inserted first into the slide port. The instrument analyzes only the first inserted chamber. Gently insert the counting slide to the end.

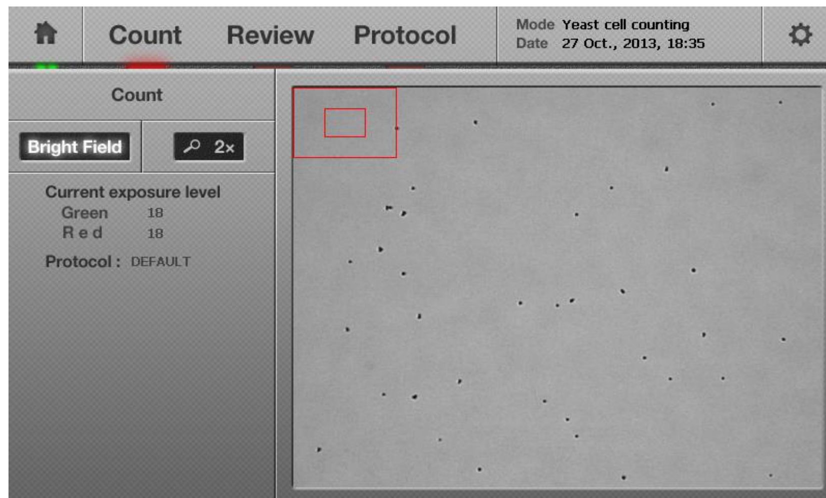
<Slide Inserting>

Note: After inserting the slide, the LUNA-FL™ only reads the first chamber. To read the second chamber, it must be taken out, rotated, and inserted again.

Note: Make sure that the counting slide is not inserted upside-down. This may lead to sample spilling and could damage the counter.

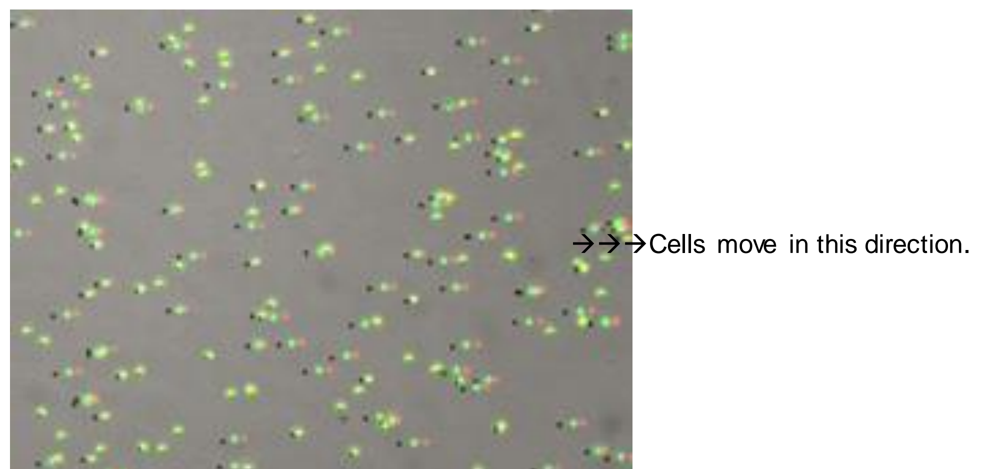
6.3.3 In the preview screen of the bright field mode, watch the yeast cells and wait for 30 sec-1 min (depending on the sample condition) in order for the cells to settle down. This step is very important because the yeast cells are very small and take some time to make a good focal plane. 2X or 4X zoom-in is helpful to focus.

<Bright Field Screen of the Yeast Counting>



Note: Skipping this step may lead to poor counting accuracy because moving cells can generate poorly aligned cell images from the bright field, green and red channel, respectively. Optimal waiting time should be determined empirically. If cells are still moving during the preview step (step 4.3.4), a longer waiting time is required. Make sure that cells do not move by observing them in the preview screen.

<Bright Field, Green, & Red Images of Unsettled Cells>

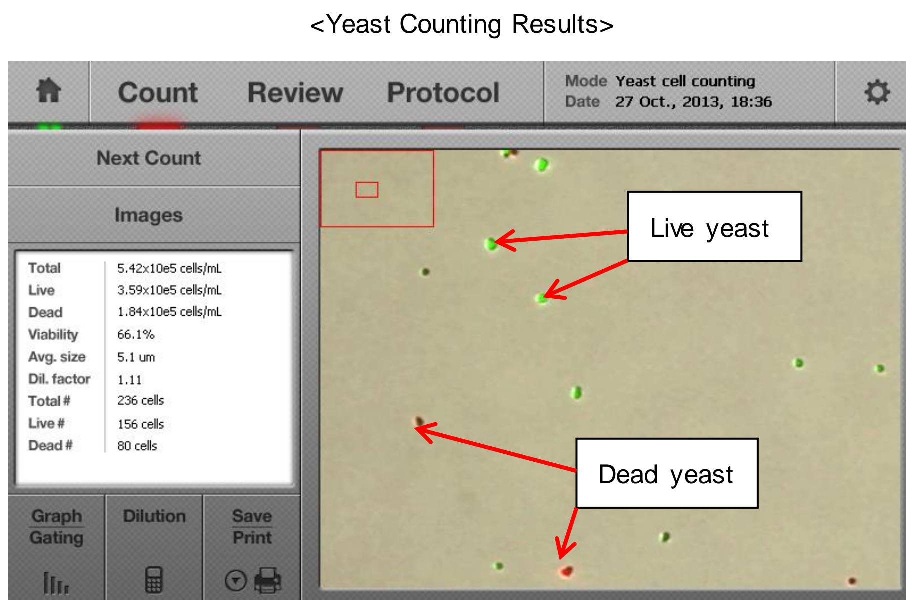


6.3.4 Check the current exposure level of green (18) and red (18) and the threshold (2) on the screen, which is the “Default Protocol” for yeast counting.

Note: Users can set the exposure level between 18 and 20 and the threshold between 1 and 5. Make sure that you start with 18 (exposure) and 2 (threshold) and then empirically determine the parameters.

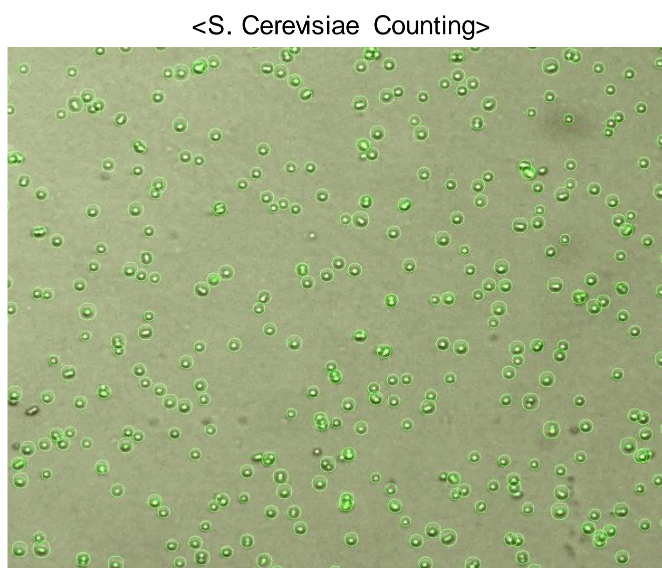
6.3.5 Touch the “Count” button.

<Yeast Counting Results>



Note: Please refer to the following counting case. S. Cerevisiae was counted perfectly with PI/FDA at exponential growth stage (Source: Courtesy provided by Vivelys).

<S. Cerevisiae Counting>

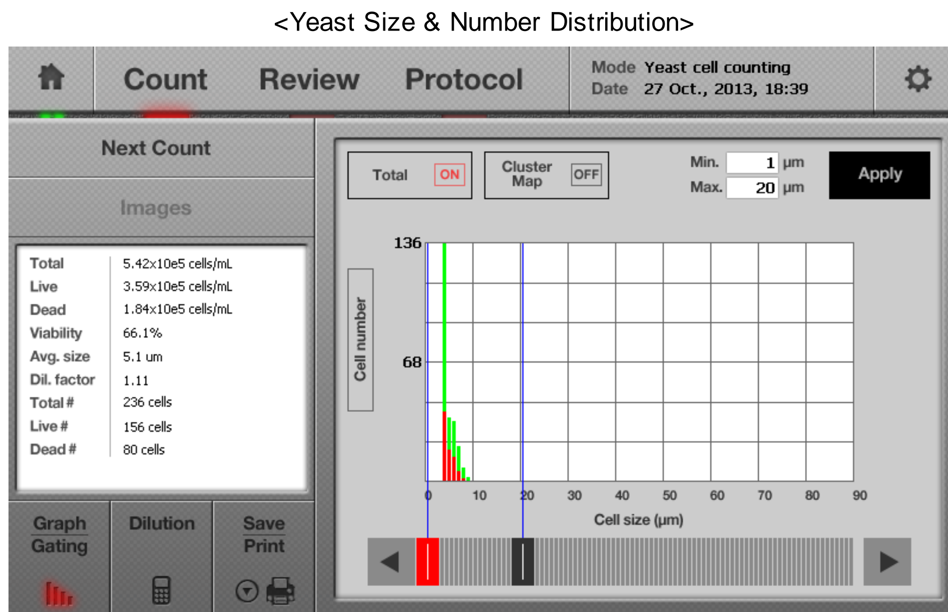


Counting Results	Counting Protocol
Total cell concentration: 7.70 x 10e6 cells/mL	Protocol name: DEFAULT

Live cell concentration: 7.70 x 10e6 cells/mL	Dilution factor: 1.11
Dead cell concentration: 0.00 x 10e0 cells/mL	Min. cell size: 3 µm
Viability: 100.0 %	Max. cell size: 60 µm
Average cell size: 9.0 µm	Size gating: 3 - 60 µm
Total cell number: 3351	Green fluorescence threshold: 5
Live cell number: 3351	Red fluorescence threshold: 5
Dead cell number: 0	

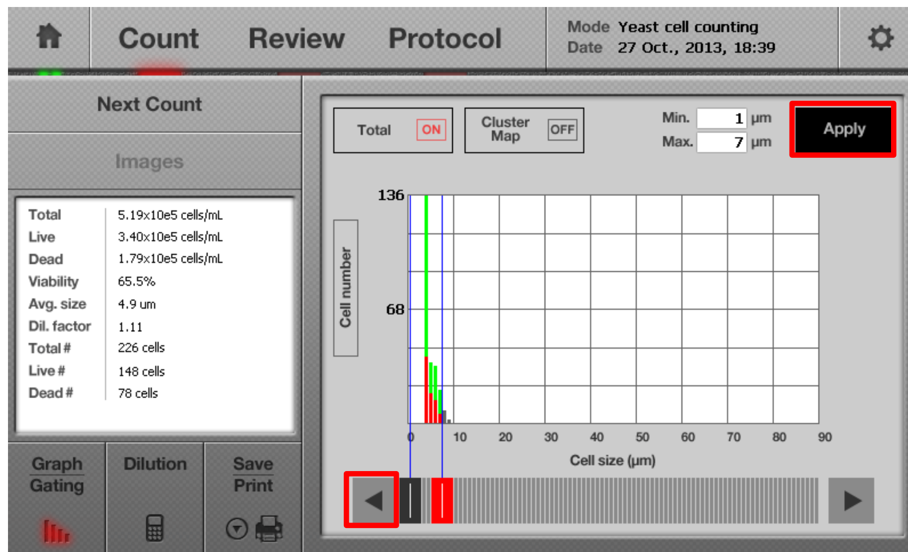
6.3.6 By clicking the “Graph/Gating” button, the screen will show the “Size & Number” distribution as shown below.

<Yeast Size & Number Distribution>



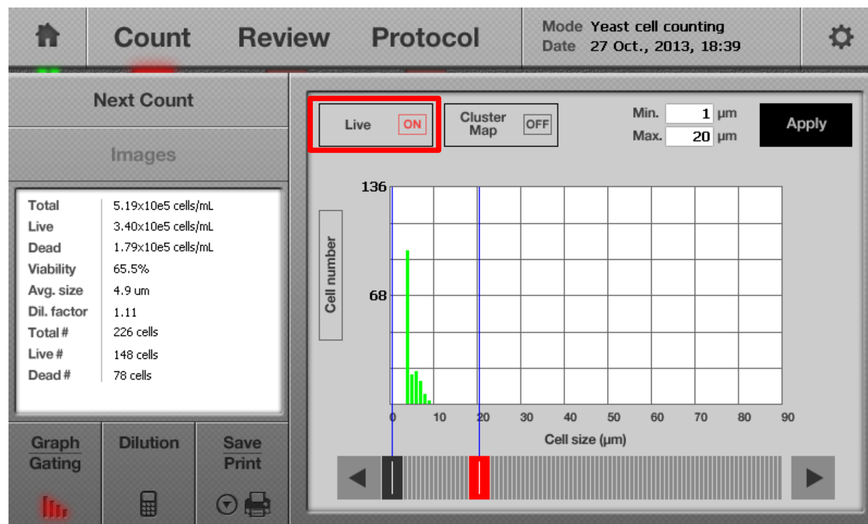
Note: By pressing the arrowhead buttons, user can define the “Min” or “Max” size of the yeast cells to include or exclude for counting. In the screen below, the “Max” size was lowered to cut off some top-sized cells, by moving the “Max” bar to the left and then clicking the “Apply”.

<Adjusting the “Max” size>

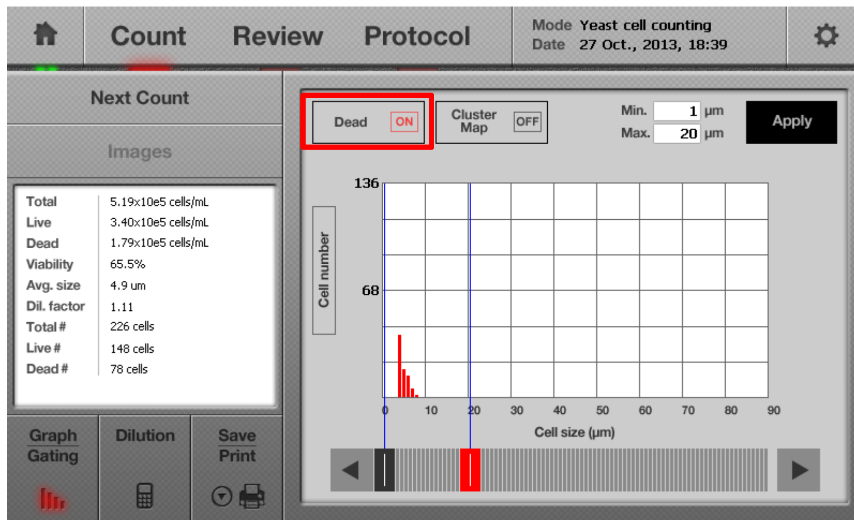


Note: By clicking the “Total/Live/Dead” button, users can see only one of the total, live, or dead cell groups as below.

<Live Cells Only>

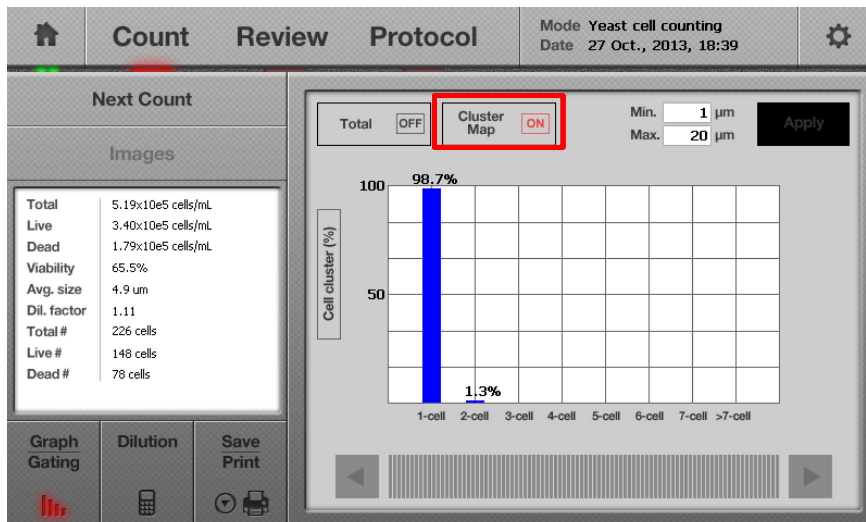


<Dead Cells Only>



Note: By clicking the “Cluster Map” button, users can see the grouping status of the “Cluster Map” as below.

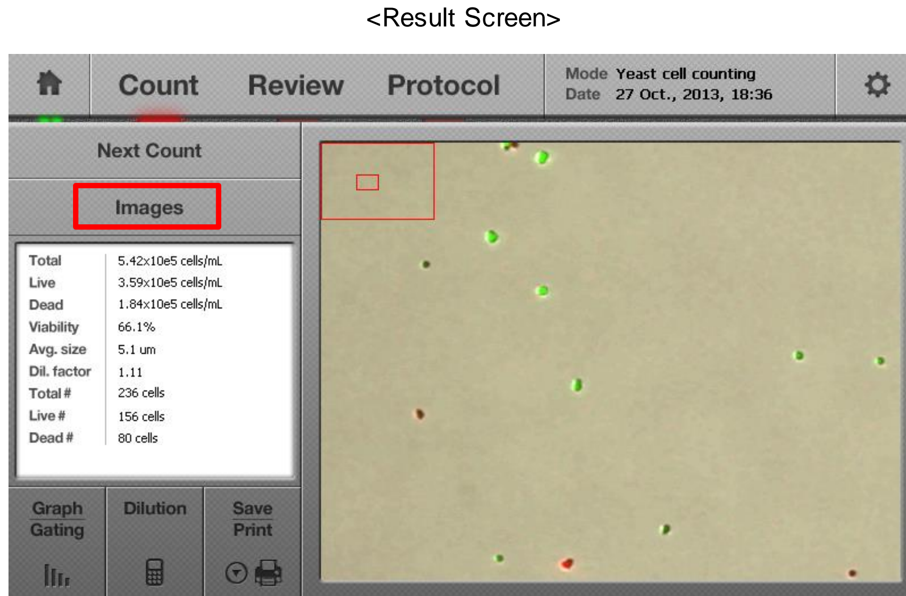
<Cluster Map>



6.4 Using the “Tag” Function

6.4.1 To verify the results after performing the counting results, press the “Images” and “Tag” buttons as shown below.

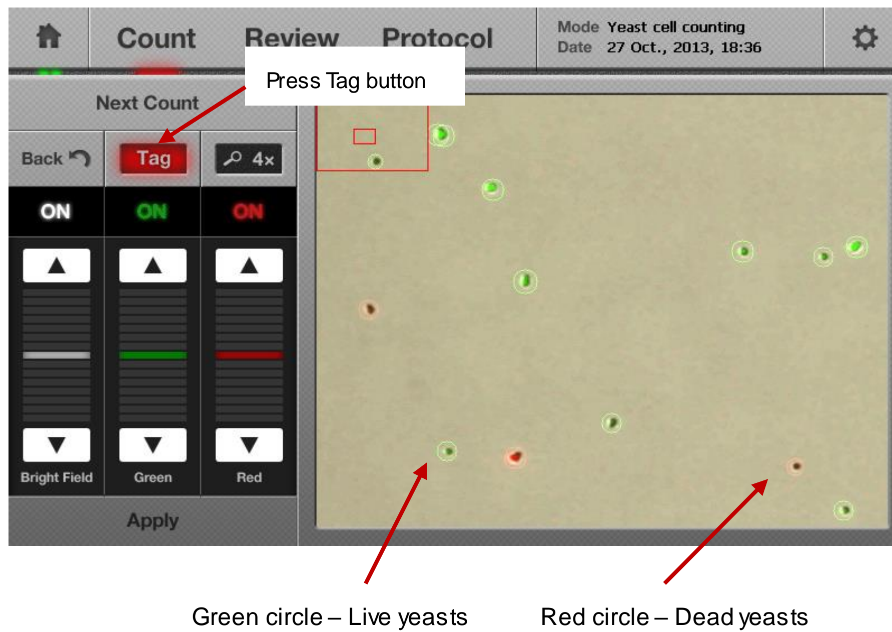
<Result Screen>



Note: This “Tag” function is one of the distinct tools of the LUNA-FL™ Automated Fluorescence Cell Counter because it helps users review and determine the accuracy on-site without depending on a computer.

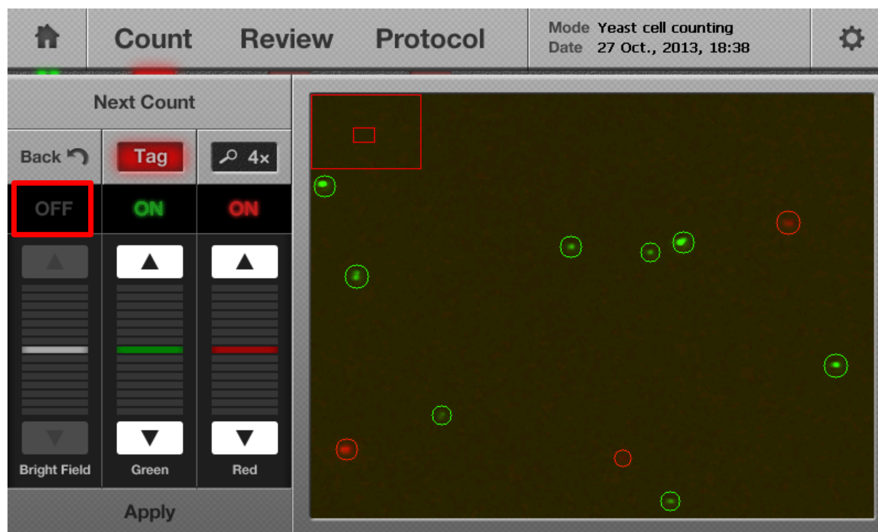
6.4.2 When touching the “Tag” button, the image on the screen will show objects surrounded by green circles. The green circles indicate live yeasts.

<Tagged Cell Image>



Note: In the scree above, the exposure level of each light channel can be changed by adjusting the arrowhead buttons.

<Turn-off of the Bright Field Channel>



6.4.3 After reviewing the accuracy of the image analysis, "Tag" button can be pressed again to remove the green circles.

6.5 Trouble Shooting

6.5.1 **(Problem 1)** The FL signal is too weak.

If your sample was grown in the YPD medium, be sure to dilute the sample at least 1:100 with Cell Dilution Buffer. The component of the YPD medium has a strong esterase activity and inhibits the uptake of FDA into the yeast cells, leading to weak fluorescence signal. For the detailed information about the effect of YPD, please read our latest application note (www.logosbio.com).

6.5.2 **(Problem 2)** The FL signal is still weak so that some yeast cells were not counted.

→ Increase the exposure up to 20.

6.5.3 **(Problem 3)** The FL signal is present, but it was not counted (circled).

→ Decrease the threshold down to 1.

6.5.4 **(Problem 4)** The background is still bright even after the dilution or washing step.

→ The Fluorescence Signal Enhancer 1 (Cat # F23213) can be used. See the following protocol.

<Using the Fluorescence Signal Enhancer 1 (Cat # F23213)>

1. Dilute yeast cells up to 1/100 with the LUNA-FL™ dilution buffer. Or, centrifuge the yeast culture and resuspend in the LUNA-FL™ dilution buffer.
2. Mix 1 μl of the Fluorescence Signal Enhancer 1 and 17 μl of the yeast sample.
3. Incubate for 10 min.
4. Add 1 μl of FDA and 1 μl of PI.
5. Incubate for 10 min.
6. Load 10 μl of the stained yeast sample on the counting slide.
7. Wait for 30 sec-1 min or until all yeast cells are immobile.
8. Touch the “Count” button.

Note: Remember that the Fluorescence Signal Enhancer 1 will be helpful, depending on the sample conditions. For example, it is helpful for exponentially growing yeasts, but, sometimes not for end-of-culture yeasts. The rapidly dividing yeasts are metabolically active and they try to pump out the activated fluorescein. The enhancers are actually inhibitors against the pumping/transporting mechanism. When used in proper conditions, the enhancers will lower background and raise the intensity of signal, thereby enhancing overall signal to noise ratio.

Note: When using Fluorescence signal Enhancer 1, set the dilution factor “1.18”.

6.6 Printing the Counting Results

Please see Section 3.8 to print the counting results.

Chapter 7 – Reviewing Previous Results

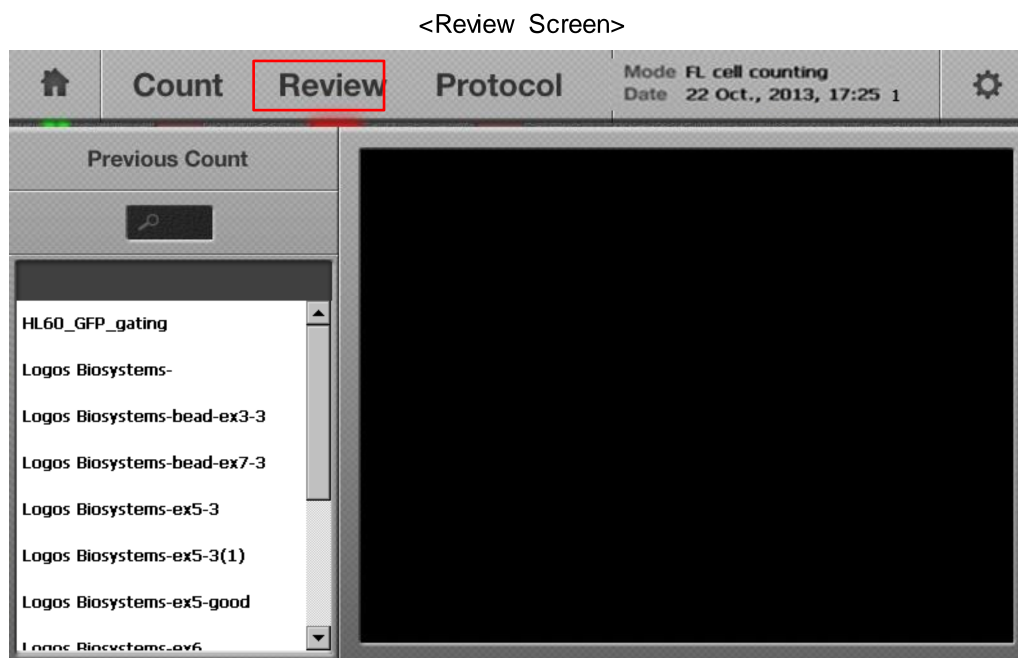
The LUNA-FL™ Automated Fluorescence Cell Counter provides an easy-to-use review function using the previous count results saved in the USB drive for further review. This “Review” function applies to all 4 counting categories.

7.1 Importing Previous Counts

7.1.1 Insert the USB drive containing the previous results into the USB port of the instrument.

7.1.2 Press the “Review” menu located at the top of the screen. A list of files on the USB drive is displayed on the left side of the screen as shown below.

<Review Screen>

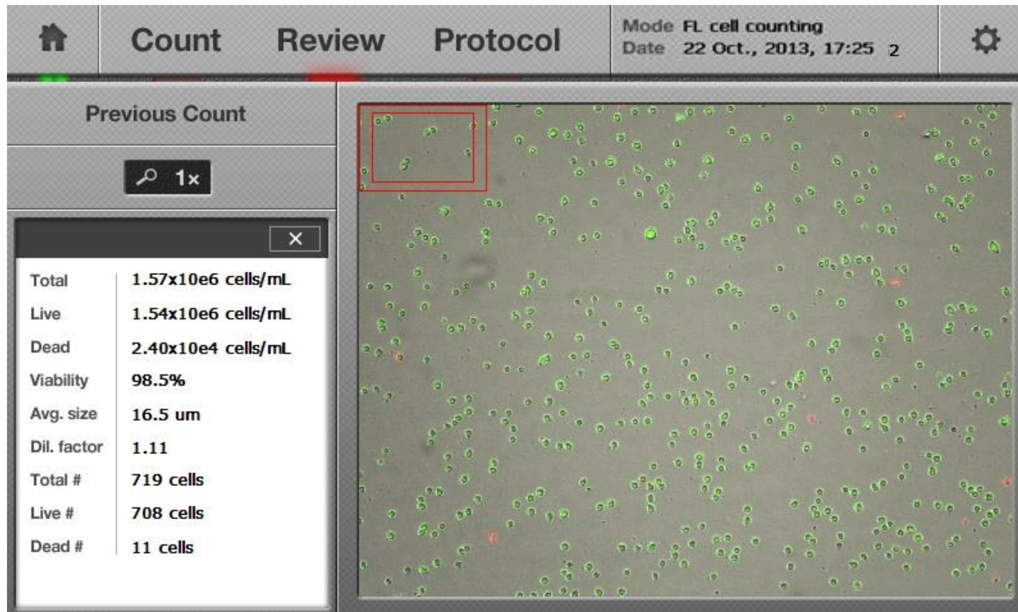


Note: If there are several saved files in the USB drive, the scroll bar can be used to navigate the file list.

7.2 Reviewing Previous Results

7.2.1 Press the file name, and the previous result will start to be imported. Both the previous image and the counting results will be displayed onto the instrument screen.

<Reviewing the previous result>



7.2.2 The previous image can be viewed at 1X, 2X or 4X by pressing the "Zoom-in" button and navigated by touching and dragging the image window.

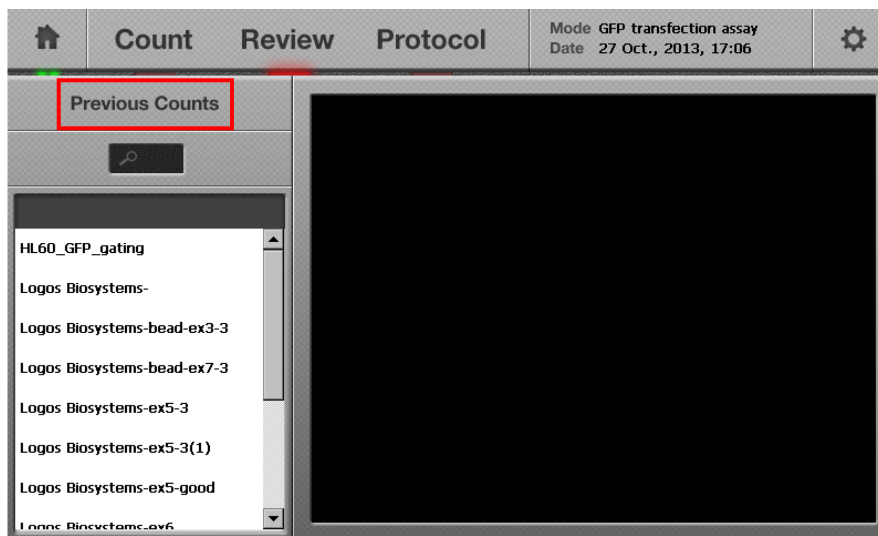
7.2.3 Press the "X (close)" button to review other previous results. Or press the "Count" menu located at the top of the screen, and the instrument will be ready to count again.

7.3 “Previous Count” button

Whenever samples are counted, results are automatically saved to the internal memory of the instrument. These data can be exported as a “.CSV” file to the USB drive for further analysis.

7.3.1 Pressing the “Previous Count” button on the left side of the “Review” menu will bring up a new window. The “Previous Count” window contains up to 1,000 previous counting results that have been saved in the memory of LUNA-FL.

<”Previous Count” Button>



<”Previous Count” Window for BF/FL/Yeast Counting>

Previous counts | Export to USB (.CSV) | Erase All

Mode	Name	Date	Total Cell	Live Cell	Dead Cell	Viability	Avg. Cell Size	Protocol
BF	1145111...	16/06/2013 13:35	6.68E+05	5.35E+05	1.34E+05	80.0%	12.1	Test Protocol
			170	136	34			
FL		15/06/2013 16:30	9.04E+05	9.04E+05	0.00E+00	100.0%	20.9	Test
			46	46	0			
BF		15/06/2013 16:28	2.91E+05	2.16E+05	7.47E+04	74.3%	15.4	Test Protocol
			74	55	19			
FL	9-20	15/06/2013 14:27	8.27E+06	8.27E+06	0.00E+00	100.0%	7.8	Test
			421	421	0			
FL		15/06/2013 14:26	8.27E+06	8.27E+06	0.00E+00	100.0%	7.8	Test
			421	421	0			
FL		15/06/2013 14:25	8.31E+06	8.31E+06	0.00E+00	100.0%	7.8	Test
			423	423	0			
FL	9-19	15/06/2013 14:24	8.31E+06	8.31E+06	0.00E+00	100.0%	7.8	Test

<"Previous Count" Window for GFP Transfection Assay>

Previous counts | Export to USB (.CSV) | Erase All | ✕

Name / Date	Total Cell	GFP(+) Cells	GFP(-) Cells	% GFP (+)	Avg. Size / Avg. Intensity	Protocol
HL60_GFP_gating	9.13E+05	8.26E+05	8.70E+04	90.5%	12.6	DEFAULT
27/10/2013 16:59	441	399	42		16769.3	
	1.00E+06	3.95E+05	6.05E+05	39.5%	10.0	DEFAULT
27/10/2013 16:53	483	191	292		6770.3	
	9.69E+05	2.96E+05	6.73E+05	30.6%	10.7	DEFAULT
27/10/2013 16:52	468	143	325		4704.0	
	9.65E+05	4.97E+04	9.15E+05	5.2%	10.5	DEFAULT
27/10/2013 16:50	466	24	442		3997.5	
	9.79E+05	1.04E+04	9.69E+05	1.1%	10.9	DEFAULT
27/10/2013 16:49	473	5	468		3766.6	
	1.29E+06	7.74E+05	5.18E+05	59.9%	8.3	DEFAULT
23/10/2013 16:49	624	374	250		19388.6	
	1.01E+06	9.71E+05	3.93E+04	96.1%	8.2	DEFAULT

7.3.2 In the window of the "Previous Count," there are 3 buttons:

1. "Export to USB (.CSV)" – Users can save the data of all previous counts into a USB memory drive, which can be handled in PC software. First insert a USB drive and then click the "Export to USB (.CSV)" button.
2. "Erase all" – All data can be removed from the memory.
3. "Close(X)" – Close this window.

<Data exporting>

Previous counts | Export to USB (.CSV) | Erase All | ✕

Name / Date	Total Cell	GFP(+) Cells	GFP(-) Cells	% GFP (+)	Avg. Size / Avg. Intensity	Protocol
HL60_GFP_gating	9.13E+05	8.26E+05	8.70E+04	90.5%	12.6	DEFAULT
27/10/2013 16:59					9.3	
					0	DEFAULT
27/10/2013 16:53					.3	
					7	DEFAULT
27/10/2013 16:52					.0	
					5	DEFAULT
27/10/2013 16:50					.5	
					9	DEFAULT
27/10/2013 16:49	473	5	468		3766.6	
	1.29E+06	7.74E+05	5.18E+05	59.9%	8.3	DEFAULT
23/10/2013 16:49	624	374	250		19388.6	
	1.01E+06	9.71E+05	3.93E+04	96.1%	8.2	DEFAULT

The data were successfully exported.

Close

Chapter 8 – Setting Up the Protocol for Counting

Users can have unique protocols for each and apply them to specific cell types. When the “Protocol” button is pressed on the main screen, a list of protocols will show up in the protocol list box.

Note: Initially, there are two protocols named as “DEFAULT” and “New protocol” in the instrument. To create a customized protocol, select “New protocol” first and edit parameters, then save as another name by pressing the “Save as” button. Because the protocol named, “DEFAULT” cannot be edited, it must be saved as another name before parameters can be adjusted.

Note: Each counting mode has its own counting parameter set.

8.1 Adjusting Parameters for Bright Field Counting

When counting is performed, the DEFAULT parameters or one of the previously set protocols will be applied to the counting.

<Bright Field Counting Parameters on the Protocol Menu>

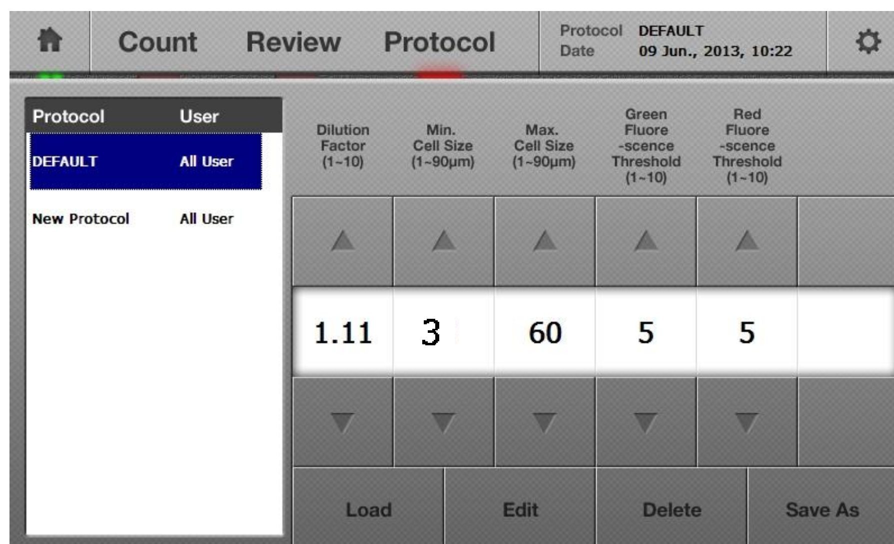
The adjustable parameters are described as follows:

Parameter	Range	Default	Description
Dilution Factor	1-100	2	The default value for the dilution factor is “2”. Users can modify this value according to the dilution of the original samples. If diluted for “A” times, users set this variable to “2 x A”. For example, 10 times diluted samples should have the dilution factor of 20 (2 x 10). Dilution factor can be adjusted either by a scale of 1 or a scale of 10 between 2 to 10 and 10-100 respectively. Dilution factor corrects the actual number of cells; thus the analyzed results are generated for the original samples. Dilution factor will help the users during repeated counting of the samples of high cell density such as fermented CHO cells. **Note**: In any case, users must combine the diluted cell samples with an equal volume of 0.4% Trypan Blue Stain (1:1 ratio) before counting.
Noise Reduction	0-10	5	This refers to the decrease of the background for counting. Higher noise reduction will not detect faint signals and weakly stained objects. Lower noise reduction means increasing the sensitivity of the objects and detecting faint signals. Adjust the noise reduction to the appropriate level. It is also useful when cells are over-stained or under-stained with Trypan Blue stain.
Live Detection Sensitivity	1-10	5	”Live Detection Sensitivity” was designed to detect the live cell having a various level of bright center. Increased “Live Detection Sensitivity” will detect more live cells. Decreased “Live Detection Sensitivity” will only detect very bright centered cells as live cells.
Roundness	30-100	60	This refers to the roundness of the objects on the image. Increasing the value requires more roundness of the objects to be included for the measurement. Meanwhile, decreasing the value includes objects that are less rounded for counting. Use this parameter when there are cell types that are not generally circular or are irregularly

			shaped.
Minimum Cell Size	1-90	3	Use this parameter to set the minimum cell size for inclusion. The unit is 1 micrometer.
Maximum Cell Size	1-90	60	Use this parameter to set the maximum cell size for inclusion. The unit is 1 micrometer.

8.2 Adjusting Parameters for Fluorescence Counting

<Fluorescence Counting Parameters on the Protocol Menu>



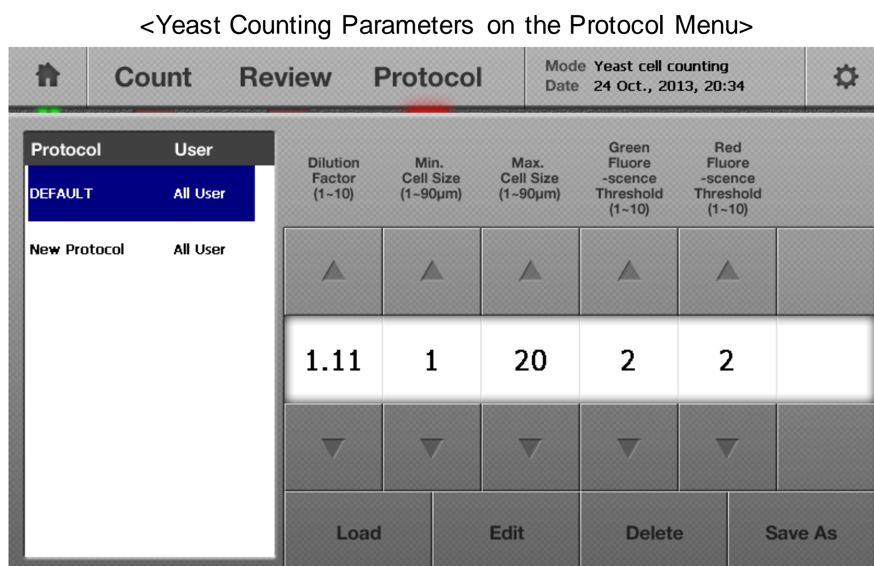
The adjustable parameters are described as follows:

Parameter	Range	Default	Description
Dilution Factor	1-10	1.11	The default value for the dilution factor is "1.11". In the protocol of the "Fluorescence Cell Counting" mode, 2 μl of the Acridine Orange/Propidium Iodide Stain (cat # F23001) is added to 18 μl of the cell sample. The dilution factor in this case is calculated as 1.11 (20/18). In case, 4 μl of the Acridine Orange/Propidium Iodide Stain is added to 16 μl of the cell sample, the dilution factor is 1.25 (20/16). When the amount of the Acridine Orange/Propidium Iodide Stain used is increased by 2 μl, the dilution factor changes from 1.11(2 μl of the Acridine Orange/Propidium Iodide Stain used), 1.25 (4 μl), 1.42 (6 μl), 1.66 (8 μl), 2.00 (10 μl), 2.50 (12 μl), 3.33 (14 μl), 5.00 (16 μl) to 10.00 (18 μl). Dilution factor corrects the actual number of cells; thus the analyzed results are generated for the original samples.
Minimum Cell Size	1-90	3	Use this parameter to set the minimum cell size for inclusion. The unit is 1 micrometer.

Maximum Cell Size	1-90	60	Use this parameter to set the maximum cell size for inclusion. The unit is 1 micrometer.
Green Fluorescence Threshold	1-10	5	Green and Red Fluorescence Threshold will determine the level of threshold during the image processing. Increased threshold will detect less cells by subtracting the background more stringently. Decreased threshold can detect more cells, but will increase the number of noise signal.
Red Fluorescence Threshold	1-10	5	

8.3 Adjusting Parameters for Yeast Cell Counting

<Yeast Counting Parameters on the Protocol Menu>



The adjustable parameters are described as follows:

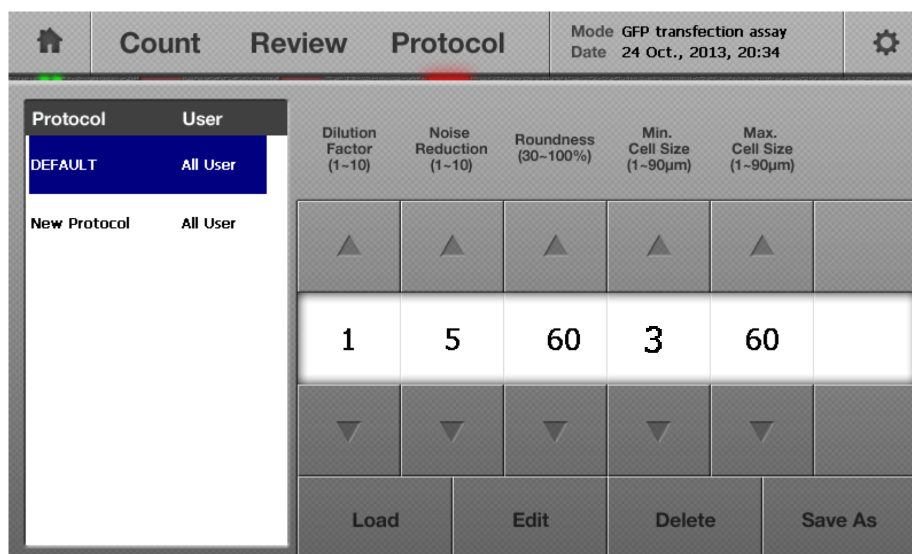
Parameter	Range	Default	Description
Dilution Factor	1-10	1.11	The default value for the dilution factor is “1.11”. When using Yeast Viability Kit 1 including Fluorescence signal Enhancer 1, set the dilution factor “1.18”(described in section 6.5.4). To perform the yeast counting, 1 µl of FDA and 1 µl of PI is added to 18 µl of the yeast sample. The dilution factor in this case is calculated as 1.11 (20/18). In case 2 µl of each FDA and PI is added to 16 µl of the cell sample, the dilution factor is 1.25 (20/16). Likewise, the dilution factor changes from 1.11(2 µl of the FDA/PI cell viability kit used), 1.25 (4 µl), 1.42 (6 µl), 1.66 (8 µl), 2.00 (10 µl), 2.50 (12 µl), 3.33 (14 µl), 5.00 (16 µl) to 10.00 (18 µl). Dilution factor corrects the actual number of cells; thus the analyzed results are generated for the original samples.
Minimum Cell Size	1-90	1	For yeast, the default min value is 1. The unit is 1 micrometer.

<table>
<tr><td>Maximum Cell Size</td><td>1-90</td><td>20</td><td>For yeast, the default max value is 20. The unit is 1 micrometer.</td></tr>
<tr><td>Green Fluorescence Threshold</td><td>1-10</td><td>2</td><td rowspan="2">Green and Red Fluorescence Threshold will determine the level of threshold during the image processing. Increased threshold will detect less cells by subtracting the background more stringently. Decreased threshold can detect more cells, but will increase the number of noise signal.

For yeast, it is recommended to start with 2 or 3, depending on the signal strength.</td></tr>
<tr><td>Red Fluorescence Threshold</td><td>1-10</td><td>2</td></tr>
</table>

8.4 Adjusting Parameters for GFP Transfection Assay

<Parameters for GFP Transfection Assay on the Protocol Menu>



The adjustable parameters are described as follows:

Parameter	Range	Default	Description
Dilution Factor	1-100	1	The default value for the dilution factor is "1". Users can modify this value according to the dilution of the original samples. If diluted for "A" times, users set this variable to "2 x A". For example, 10 times diluted samples should have the dilution factor of 20 (2 x 10). Dilution factor can be adjusted either by a scale of 1 or a scale of 10 between 2 to 10 and 10-100 respectively. Dilution factor corrects the actual number of cells; thus the analyzed results are generated for the original samples. Dilution factor will help the users during repeated counting of the samples of high cell density such as fermented CHO cells. **Note**: In any case, users must combine the diluted cell samples with an equal volume of 0.4% Trypan Blue Stain (1:1 ratio) before counting.
Noise	0-10	5	This refers to the decrease of the background for

Reduction			counting. Higher noise reduction will not detect faint signals and weakly stained objects. Lower noise reduction means increasing the sensitivity of the objects and detecting faint signals. Adjust the noise reduction to the appropriate level. It is also useful when cells are over-stained or under-stained with Trypan Blue stain.
Roundness	30-100	60	This refers to the roundness of the objects on the image. Increasing the value requires more roundness of the objects to be included for the measurement. Meanwhile, decreasing the value includes objects that are less rounded for counting. Use this parameter when there are cell types that are not generally circular or are irregularly shaped.
Minimum Cell Size	1-90	3	Use this parameter to set the minimum cell size for inclusion. The unit is 1 micrometer.
Maximum Cell Size	1-90	60	Use this parameter to set the maximum cell size for inclusion. The unit is 1 micrometer.

8.5 Managing Protocols

To manage protocols, use the buttons described below.

Button	Description
Load	Use this button to load one of the saved protocols to apply for counting or adjusting the parameters.
Edit	After selecting a protocol, parameters can be adjusted by pressing this button. Arrow buttons located over and under the parameter will be activated. Press the arrow buttons to change the values for each parameter. When no protocol is selected, this button is inactivated. If you “Load” a new protocol after you “Edit” a protocol, the changed parameters will be automatically saved to the same protocol name.
Delete	After selecting one of the protocols on the screen, press this button to remove it from the instrument. When no protocol is chosen or loaded, this button is inactivated.
Save as	After adjusting the parameters, press this button to save a new protocol.

	After saving, users can load the protocol for future counting.

Note: The LUNA-FL™ Automated Fluorescence Cell Counter is provided with a factory setting protocol named "DEFAULT". This "DEFAULT" protocol cannot be edited or deleted. The parameters in the "DEFAULT" protocol have been tested with many different cell types and can be used for a wide variety of cells without changing parameters.

Chapter 9 – Maintenance and Troubleshooting

9.1 Cleaning

Generally, the LUNA-FL™ Automated Fluorescence 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 LUNA-FL™ cell counter. Be sure to turn off the LUNA-FL™ cell counter and disconnect the power cable before cleaning and performing any other maintenance. Ensure that water and other solutions do not enter any part of the instrument during cleaning.

9.1.1 Cleaning the case

With a soft, 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.

9.1.2 Cleaning the touch screen

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

9.1.3 Decontaminating with alcohol

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

Note: Do not use an abrasive or a bleach solution that can cause scratches on the outer case or the touch screen.

9.2 Changing the Battery

The battery of the LUNA-FL™ Automated Fluorescence Cell Counter is expected to last for 10 years. However, if the date/time changes or slows with unknown causes, it may indicate that the battery is weakening or running out. To change the battery, contact the service personnel of the instrument supplier in your country. Do not attempt to change the battery in any event. Disassembling the instrument voids the product warranty.

9.3 Calibrating the Counter

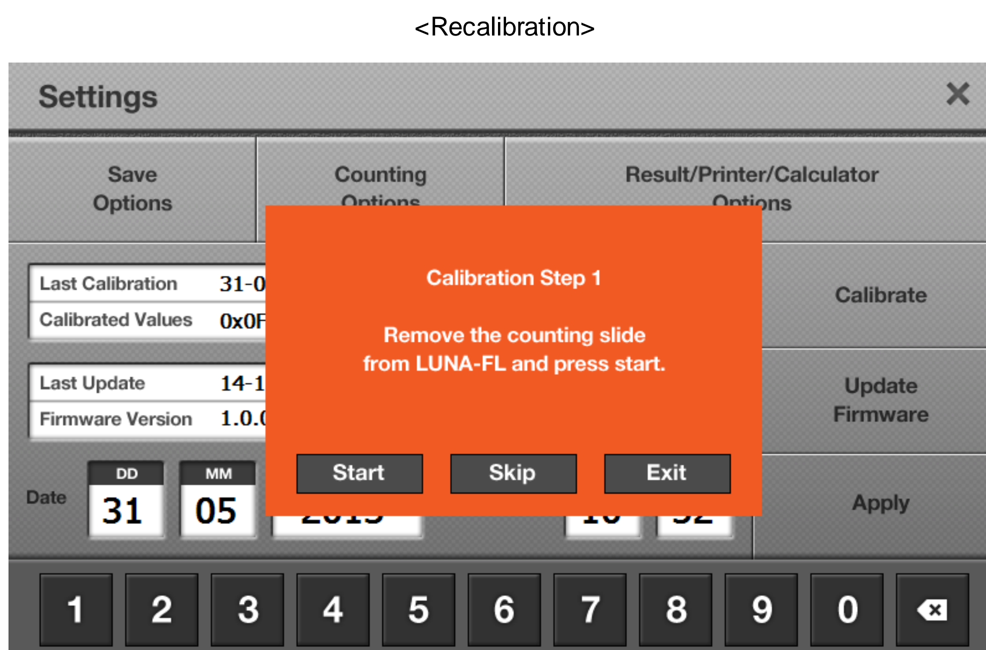
In general, recalibration of the LUNA-FL™ Automated Fluorescence Cell Counter is not necessary since it is pre-calibrated during manufacture. However, if needed for the installation or operation qualification, please follow the procedure below.

9.3.1 Turn on the instrument to show the Start-Up screen on the display. If the instrument is already turned on, please turn off and on, and then go to the "Start-Up" screen. Select the "Bright Field Cell Counting" or "Fluorescence Cell Counting" mode. The calibration can be performed at either mode.

Note: Be sure to focus the instrument properly before calibration. Use the calibration bead to for the focus adjustment.

9.3.2 Go to the "Settings" menu. Press the "Calibration" button and a message box will appear as shown below.

<Recalibration>

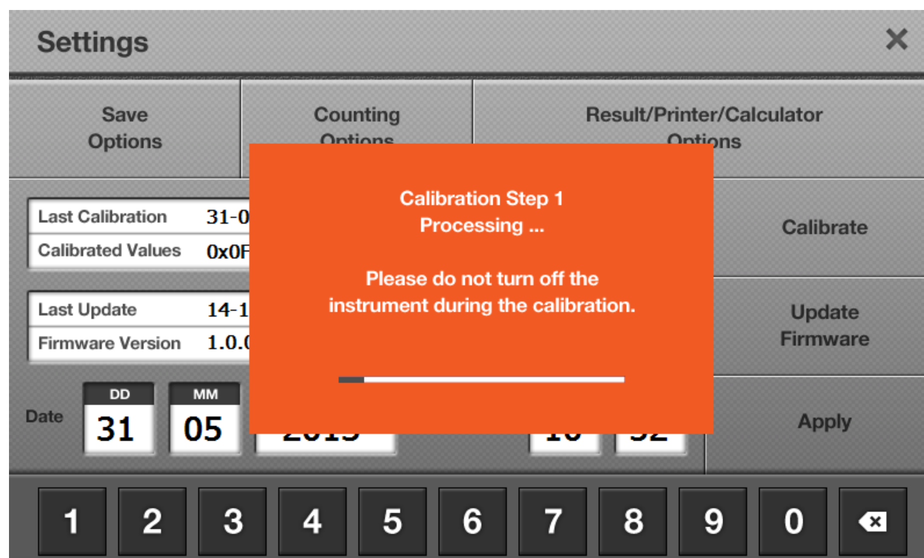


Note: The recalibration consists of 3 steps for bright field calibration, custom staining calibration, and fluorescence calibration. Depending on the requirements, each step can be performed or skipped.

9.3.3 "Calibration Step 1" is necessary for bright field calibration. It can be performed by pressing the "Start" button, or skipped by pressing the "Skip" button. When you click the "Exit" button, you can quit this procedure at any time.

Note: It is very important to remove the counting slide prior to start the calibration step 1. If the calibration step 1 is performed without removing the counting slide, cell counting result will be inaccurate.

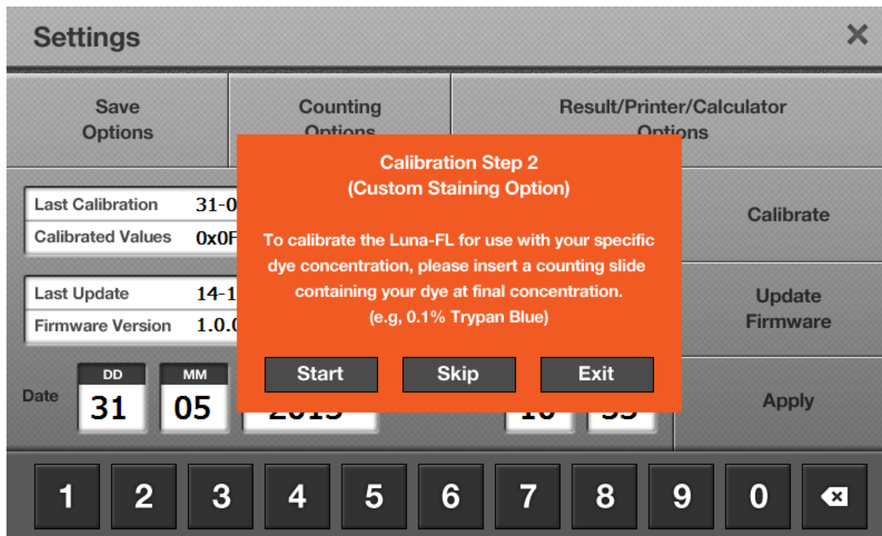
<Calibration Step 1>



Note: Never turn off the LUNA-FL™ cell counter during calibration, which could lead to a significant technical failure.

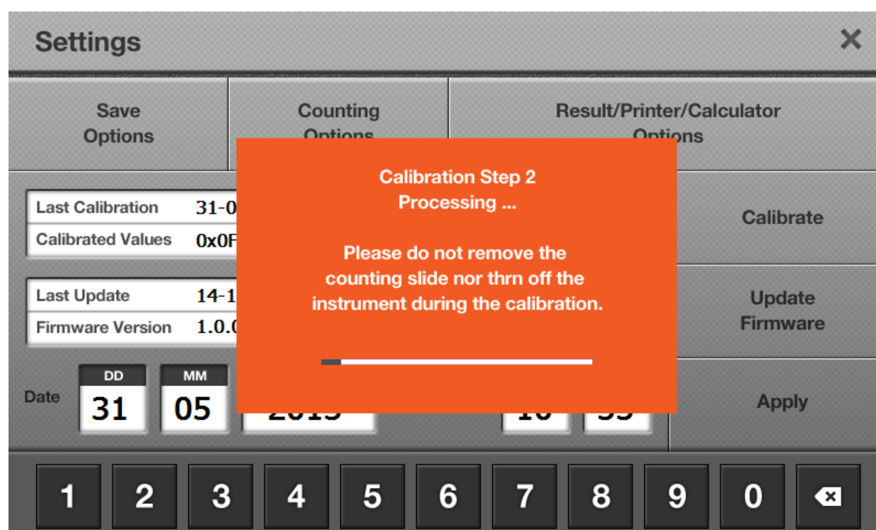
9.3.4 “Calibration Step 2” is necessary for the “Custom Staining Calibration.” From this custom calibration, users can use their own stains for cell counting. This is related to Section 2.6 Counting Options.

<Calibration Step 2-1>



Note: To perform the "Custom Staining Calibration", users must be careful in preparing the staining solution. For example, when users want to count cells under 0.1% Trypan Blue concentration, first, mix 5 µl of 0.4% Trypan Blue Stain with 5 µl of PBS to prepare 10 µl of 0.2% concentration. Second, mix 10 µl of 0.2% concentration with another 10 µl of PBS, to prepare 20 µl of 0.1% concentration, from which 10 µl of 0.1% is loaded into one counting chamber to perform 0.1% Custom Staining Calibration. To count cells under 0.1% Trypan Blue concentration, users can mix cells with 0.2% Trypan Blue Stain at a 1:1 ratio.

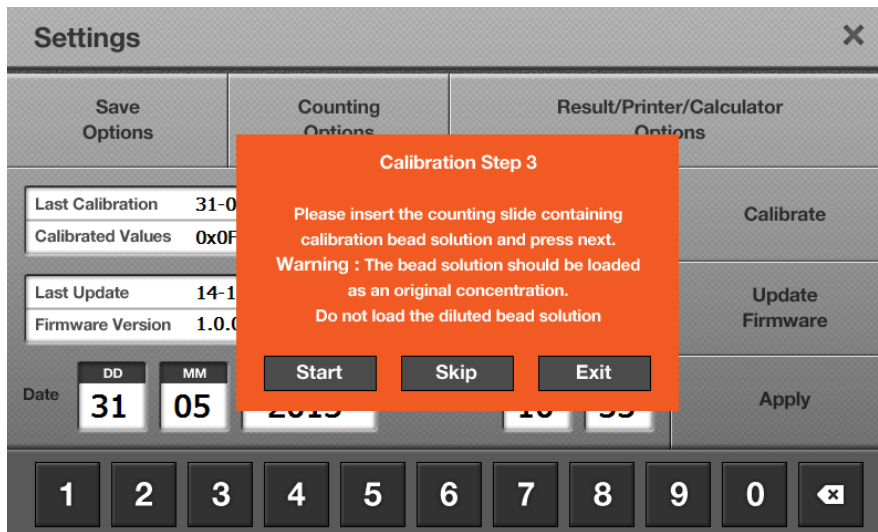
<Calibration Step 2-2>



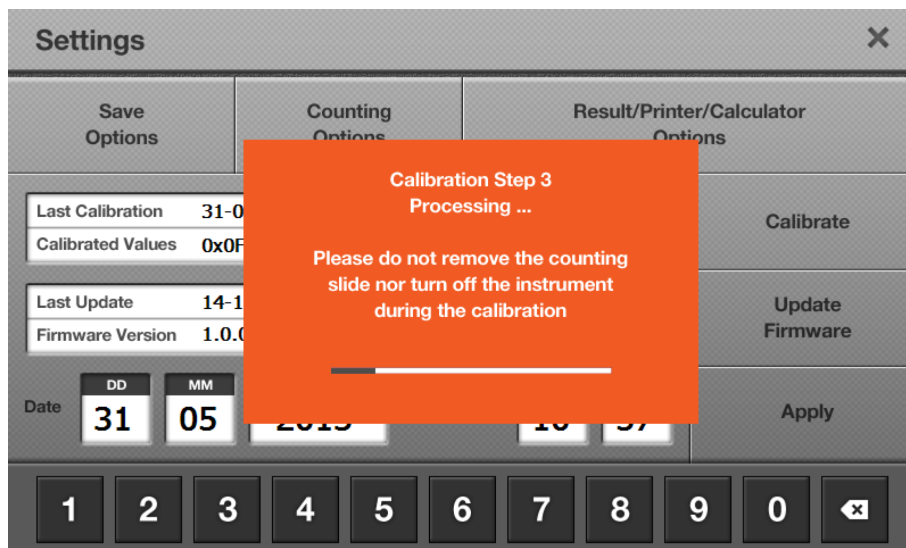
Note: Never turn off the LUNA-FL™ cell counter during calibration, which could lead to a significant technical failure.

9.3.5 “Calibration Step 3” is necessary for “Fluorescence Counting Calibration.” Fluorescence standard beads are supplied by Logos Biosystems or local distributors. Load 10 µl of fluorescence calibration beads into one of the counting chambers and wait for about 1 min in order for cells to completely settle down. Insert the counting slide and then press the “Start” button. Make sure to load the original standard solution - do not dilute it.

<Calibration Step 3-1>



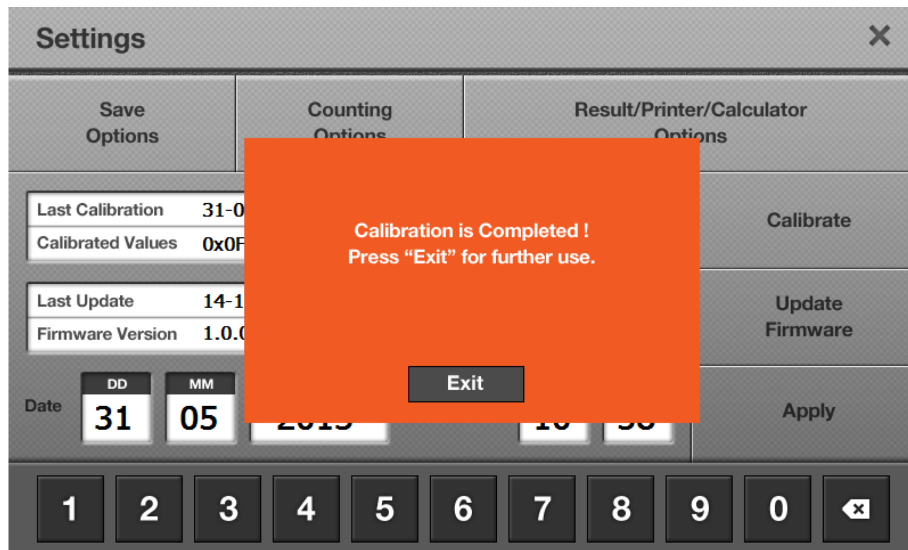
<Calibration Step 3-2>



Note: Never turn off the LUNA-FL™ cell counter during calibration, which could lead to a significant technical failure.

9.3.6 When the three calibration steps are done successfully, users will see the message box as below.

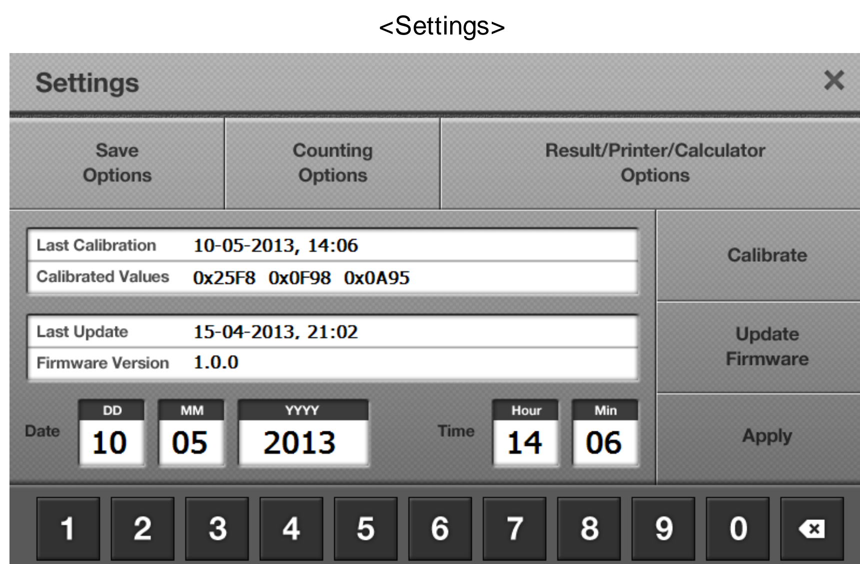
<Successful Calibration>



9.4 Updating Firmware

9.4.1 If the instrument is already turned on, please turn off and on, and then go to the "Start-Up" screen. Press the "Settings" button and then the information of the last update and current firmware version will be shown as below.

<Settings>



9.4.2 Check the firmware version and download the latest version from the LUNA-FL™ website at www.logosbio.com if necessary. Save the file in a USB drive.

Note: The firmware, consisting of 1 file, must be saved into the root directory of the USB drive.

9.4.3 Insert the USB drive into the USB port on the instrument. Then press the "Update Firmware" button.

9.4.4 When the display asks you to update, press the "Start" button. The process will take a few minutes for completion.

9.4.5 When the update is finished, press the "Restart" button to reboot the instrument.

Note: The firmware update does not remove the user-created "Protocols" previously saved in the instrument. Thus, after the update, all previous protocols are available for cell counting.

Note: Re-calibration is not necessary after a firmware update.

9.5 Troubleshooting

Problem	Possible Cause	Solution
Inaccurate cell count	Unfocused cell image	Ensure that the cell image is appropriately focus by using the focus knob. You may use the "Zoom-in" button when adjusting the cell image. Make sure that live cells have bright centers and dead cells have dark/blue centers.
	Clumped cells	Make sure that cells are not clumped. The more single cells, the better counting results.
	Cell concentration range	The cell concentration range of the instrument is preferably 5 x 10^4 - 1 x 10^7 cells/ml. Make sure that the sample is in this range. The sample may need to be concentrated or diluted.
	Counting slide insertion	Ensure that the counting slide is inserted completely into the instrument. When the slide is inserted, a soft click can be heard.
	Sample loading	If the counting slide is over- or under-loaded with the sample, it may affect counting results. The optimal amount 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.
	Damaged counting surface	Make sure that the counting area of the slide is transparent before loading the sample. Wear gloves while handling the slide.
	Floating cells	After loading the sample in the counting chip, wait for about 1 min to allow the cells to settle down. Time required for each cell type can vary and should be determined empirically.
	Omitted dyes	In the "Fluorescence Cell Counting" mode, cells cannot be counted without fluorescent nucleic acid binding dyes, e.g., AO and PI.
	Too low or too high fluorescence setting	Make sure fluorescence settings in the preview screen are in a reasonable range.
	Inaccurate calibration	Every LUNA-FL™ instrument has been pre-

		calibrated during the manufacturing process. In the rare event when calibration is wrong, perform re-calibration as explained in Section 7.3.
	Low precision range	The precision of cell counting results is dependent on the number of cells actually counted. The more cells that are counted, the more precise the result. Although LUNA-FL™ can count as low as 5 x 10^4 cells/ml, cell concentration higher than 2 x 10^5 cells/ml is required for 10% or lower Coefficient for variation (CV).
Data transfer and saving	Incorrect 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 incompatible 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 rear side of the instrument. Please contact service engineer if the calibration fails repeatedly.
	Incorrect 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 firmware version	Delete previous versions from the USB drive before downloading new firmware.
	Incorrectly saved or damaged firmware	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 10 – Ordering Information

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

	Cat #	Product	Quantity
Counter	L20001	LUNA-FL™ Automated Fluorescence Cell Counter	1 unit
Slides	L12005	PhotonSlide™, 50 Slides	1 box
	L12006	PhotonSlide™, 500 Slides	10 boxes
	L12007	PhotonSlide™, 1000 Slides	20 boxes
	L12001	LUNA™ Cell Counting Slides, 50 Slides	1 box
	L12002	LUNA™ Cell Counting Slides, 500 Slides	10 boxes
	L12003	LUNA™ Cell Counting Slides, 1000 Slides	20 boxes
	L12008	LUNA™ Reusable Slide	1 unit
	L12010	LUNA™ Reusable Slide Coverslips	10 units
Beads	B13101	LUNA™ Standard Beads	2 x 1 mL
	F23102	LUNA™ Fluorescence Calibration Beads	1 x 0.5 mL
Reagents	F23001	Acridine Orange/Propidium Iodide Stain	2 x 0.5 mL
	F23002	Acridine Orange Stain	2 x 0.5 mL
	F23003	Propidium Iodide Stain	2 x 0.5 mL
	F23202	Yeast Viability Kit 1	1 kit
	F23004	Propidium Iodide Stain for Yeast	2 x 0.5 mL
	F23211	Fluorescein Diacetate Stain	2 x 0.5 mL
	F23213	Fluorescence Signal Enhancer 1	2 x 0.5 mL
	T13001	Trypan Blue Stain, 0.4%	2 x 1 mL
	L13002	Erythrosin B Stain	2 x 1 mL
	F23212	Cell Dilution Buffer	5 x 20 mL
	F53002	Cell Dilution Buffer II	5 x 20 mL
Accessories	P10001	LUNA™ Printer	1 unit
	P12001	LUNA™ Printer Paper - thermal, 700 prints	3 x 2 rolls
	P13001	LUNA™ Printer Cleaning Pen	1 unit
	U10005	USB Drive, 16 GB	1 unit

Chapter 11 – Purchaser Notification

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 unus ed, the Company will accept return of the product with a full refund.

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

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

Logos Biosystems, Inc. ("Company") will not claim any consideration against the purchaser of infringem ent 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.

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 Peri od 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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