

# LUNAII yf<sup>TM</sup>

Automated Yeast Cell Counter

## User Manual



## DISCLAIMER

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

## TRADEMARKS

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



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



Protective earth (Ground)

## FCC COMPLIANCE

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

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# Safety Precautions

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

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

# General Guidelines

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

1. Hold PhotonSlides™ by the edges to avoid touching the optical surface. Take care that the optical surfaces of the slide do not become smudged, damaged, or contaminated.
2. When staining yeast cells with cell permeant nucleic acid dyes, perform counting after ten minutes of mixing samples for accurate cell viability measurements. If necessary, count your sample twice (duplicate readings) and take an average.
3. As the LUNA-II YF™ is calibrated before shipping, recalibration before use is not necessary. See Section 2.3.4: Settings: Background Calibration to see when background calibration is necessary.

## Environmental Conditions for Operation

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

# Chapter 1 – Introduction

## 1.1 Product Overview

Brewing and winemaking use yeast to convert carbohydrates into alcohol in anaerobic conditions, a process called fermentation. The quality and quantity of yeast cells “pitched” to start the fermentation process greatly affects the aroma, clarity, and flavor of the final product. Too few yeast cells can result in soapy and chemical flavors due to byproducts created by stressed and overworked yeast cells, incomplete fermentation, or unwanted bacterial infections. Although more yeast pitched can lead to cleaner flavors, the variability in quantity of yeast added can make reproducing quality and flavor between batches difficult. Some brewers and winemakers pitch by volume or weight, a highly variable and inaccurate way of estimating the number of yeast added. Hemocytometers and microscopes are the main tools used to analyze yeast cell count and viability, a time consuming process with a high level of user-user variability.

The LUNA-II YF™ Automated Yeast Cell Counter is a fully automated solution for yeast cell counting and viability analysis. An autofocusing liquid lens and a proven counting algorithm make yeast cell counting a simple, quick, and reproducible task. Simply prepare a yeast sample solution and the LUNA-II YF™ does the rest, doing away with the subjectivity and time expenditure of manual cell counting.

The LUNA-II YF™ produces:

- the total number of cells per mL,
- the number of live and dead cells per mL,
- the viability of cells (% live cells to total cells),
- cell images (optional: labeling live and dead cells as green and red circles, respectively).

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

Both reusable and disposable slides can be used with the LUNA-II YF™. Designed for cost-efficient and accurate cell counting, the LUNA™ Reusable Slide combines the economy of manual cell counting with the speed, accuracy, and convenience of automated cell counting. The disposable PhotonSlides™ are made with optical quality materials with low autofluorescence to allow for optimal fluorescence detection. PhotonSlides™ maintain the highest standard of cell counting accuracy and offer the ultimate counting experience with no mess or cleanup.

## 1.2 Key Features

Key Features	Description
Dual fluorescence optics	The LUNA-II YF™ uses two fluorescence channels to produce accurate cell viability information. The intensity of each light source can be adjusted independently.
Compact, space-efficient design	Lightweight and compact, the LUNA-II YF™ maximizes space and may be used on a laboratory bench or in a biosafety cabinet.
Accuracy & precision	Sophisticated optical components and a proven counting algorithm provide accurate and reproducible results.
Autofocusing	A non-mechanical liquid lens efficiently and reliably autofocuses on cells, removing human error and enabling accurate cell counting.
Easy-to-operate user interface	Straightforward and intuitive software allows users to capture and analyze cell count and viability data with ease.
Counting time	With manual focusing, you are 10 seconds away from your data. With autofocusing, a mere 15 seconds.
Cell concentration range	Cell concentrations ranging from $5 \times 10^4$ to $1 \times 10^7$ cells/mL are easily analyzed.
Simple dilution calculations	Onboard software calculates dilutions for users.
Onboard memory	Up to 1000 count results can be saved directly to the LUNA-II YF™.
Customizable protocols	Up to 300 unique protocols can be set and used.
Data reports	Detailed PDF files complete with cell count and viability data, images, and histograms can be saved to an external drive.

## 1.3 Product Contents

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

Component	Quantity
LUNA-II YF™ Automated Yeast Cell Counter	1
Power Cord with AC Adapter	1
PhotonSlide™, 50 Slides	1 box
Acridine Orange/Propidium Iodide Stain	2 x 0.5 mL
Cell Dilution Buffer II	1 x 25 mL
USB Drive, 16 GB	1

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

## 1.4 Product Specifications

LUNA-II YF™ Automated Yeast Cell Counter Specifications	
Instrument Type	Benchtop cell counter
Dimensions (W x D x H)	16 x 18 x 28 cm (6.3 x 7.0 x 11.0 in)
Weight	1.8 kg (3.9 lb)
Cell Concentration Range	$5 \times 10^4$ - $1 \times 10^7$ cells/mL
Cell Viability Range	0 - 100%
Image Type	TIFF, 1.1 MP
Processing Time*	10'' (manual focusing) or 15'' (autofocusing) seconds at $\sim 1 \times 10^6$ cell/mL

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

''This is the minimum processing time for each focusing option at the specified concentration of *Saccharomyces cerevisiae*.

PhotonSlide™ Specifications	
Material	Poly(methyl methacrylate) (PMMA)
Dimensions (W x D x H)	25 x 75 x 2.4 mm
Chamber Depth	100 $\mu$ m
Chamber Volume	10 $\mu$ L

## 1.5 Product Description

### 1.5.1 Front View of the LUNA-II YF™

The front of the LUNA-II YF™ has a wide touchscreen, a power button, a slide port to insert slides and a USB port for easy data transfer.



### 1.5.2 Rear View of the LUNA-II YF™

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





# Chapter 2 – Setting up

## 2.1 Installation

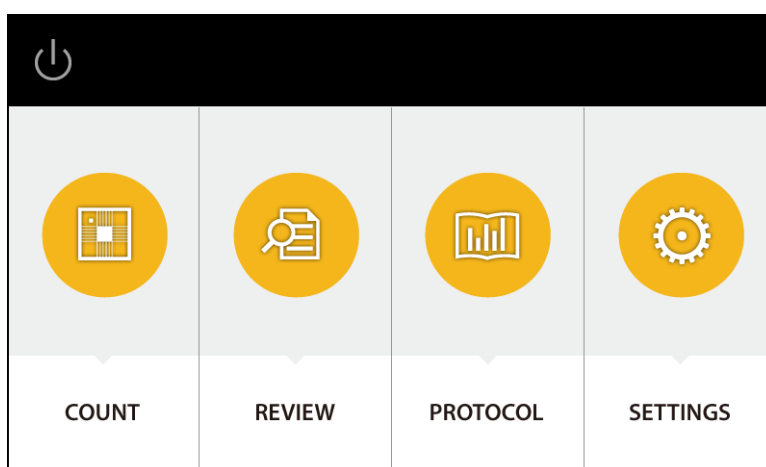
Place the LUNA-II YF™ on a clean and level surface. Connect the power cord to the AC adapter. Connect the power cord to an electrical outlet after checking the outlet configuration in your local area.

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

## 2.2 Startup/Main Menu

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

The main menu has a power icon and four options: **COUNT**, **REVIEW**, **PROTOCOL**, and **SETTINGS**.



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




## 2.3 Settings

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

Select **SETTINGS** from the main menu.

The Settings screen displays:

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

		<b>Settings</b>	Protocol FDA-PI
			Date 07 MAR 2016 16:08
<div>Save Options</div> <div>Dilution Calculator Options</div> <div>Date / Time</div>			
 Background Calibration	Last calibration update date	07 MAR 2016 16:08	
	Calibration value	LED:56, G:20, R:40, X:0, Y:1	
 Software Update	Last S/W update date	07 MAR 2016 16:02	
	Current S/W version	1.0.0 / 2	

Settings options:




- **2.3.1 [Save Options]** Preset the types of files saved and customize the file name prefix.
- **2.3.2 [Dilution Calculator Options]** Select the concentration value to use for dilution calculations.
- **2.3.3 [Date/Time]** Adjust the date and time of your instrument for record keeping purposes.
- **2.3.4 [Background Calibration]** Perform background calibrations with each software update.
- **2.3.5 [Software Update]** Update software to the most recent version.

### 2.3.1 Settings: Save Options

Save Options allows the user to set the type of file(s) saved and the default file name prefix used for subsequent counts.

Press **Save Options** in the Settings screen.

There are three saving options.

Default Save		Description
Analyzed Image		Tagged image of live and dead cells
Raw Image		Untagged images of cells in each channel
Report		PDF report with count data and histograms

Select the desired saving options by pressing the boxes in front of each option. Selected boxes are yellow. Unselected boxes are black.

Using the onscreen keyboard, enter the desired default file name prefix.

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

Press **Save** to set the default save options.

<

Default Save : ☒ Analyzed Image ☐ Raw Image ☐ Report

File name prefix :

1234567890-

QWERTYUIOP↩

ASDFGHJKL

⬆ZXCVBNM

Space

Add Date / Time

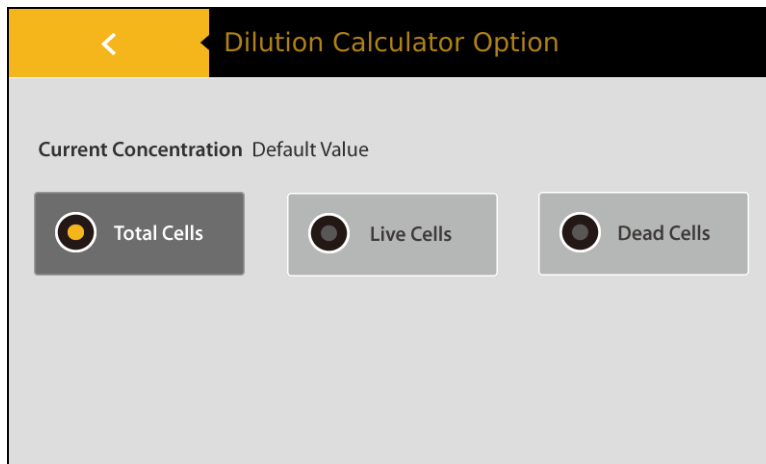
Save

Press < to return to the Settings screen.

### 2.3.2 Settings: Dilution Calculator Options

Users may use the onboard dilution calculator to compute dilutions for subsequent experiments. When calculating dilutions, the calculations can be made with total cell concentration, live cell concentration, dead cell concentration, or a custom cell concentration. The Dilution Calculator Option allows the user to select which concentration value (total, live, or dead) to use for subsequent dilution calculations.

Press **Dilution Calculator Options** in the Settings screen.



Select the desired value. The selection will be marked with a yellow circle.

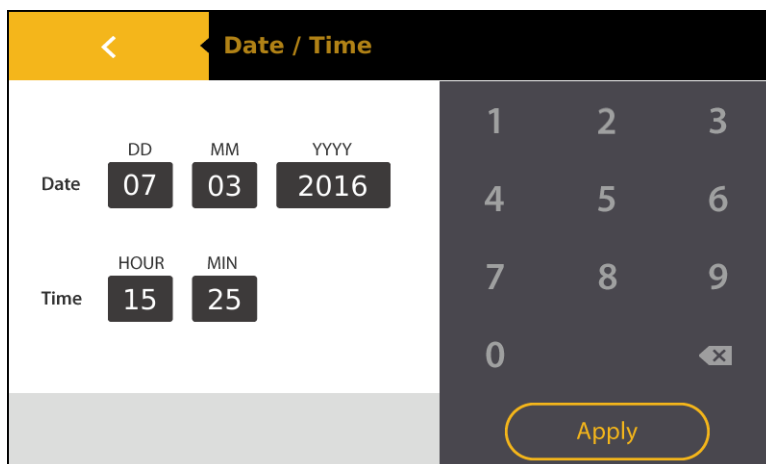
Press < to return to the Settings screen.

### 2.3.3 Settings: Date/Time

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

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

Select the desired field to delete the existing value.



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

Press < to return to the Settings screen.

## 2.3.4 Settings: Background Calibration

Background calibration adjusts for the specific shade of the stain used for counting and is a prerequisite for the successful detection of cells. As the LUNA-II YF™ is calibrated before shipping, recalibration before use is unnecessary. Users must recalibrate the background after each software update.

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

Put 10 µL LUNA™ Fluorescence Calibration Beads into the chamber of a new PhotonSlide™ or a LUNA™ Reusable Slide. Wait for 30 seconds to 1 minute for the beads to settle down.

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

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

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

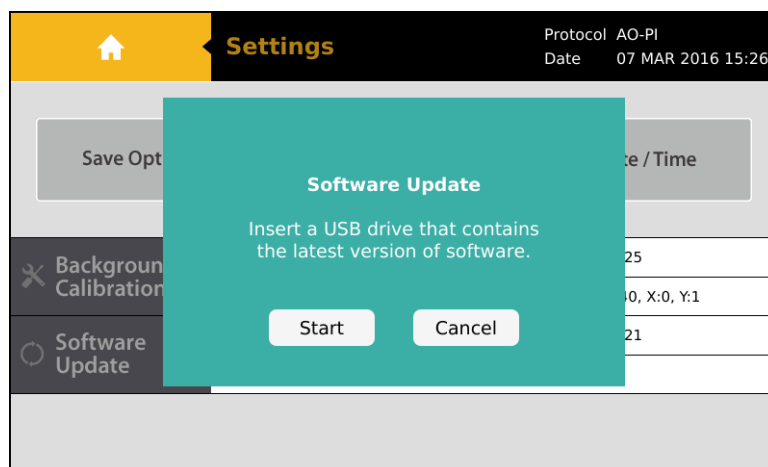
The background calibration value and date will have changed in the Settings Screen.

## 2.3.5 Settings: Software Update

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

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

Press **Software Update** in the Settings screen.



Insert the USB drive with the downloaded file into the USB port.

Press **Start**. Do not turn the instrument off during the update.

The date and version of the last software update will change automatically in the Settings screen.

**! Important!** Users must recalibrate the background after each software update (see Section 2.3.4: Settings: Background Calibration).

## Chapter 3 – Protocol Settings

The LUNA-II YF™ provides two default protocols that can be used for most common yeast cells. Users may create and save up to 300 unique protocols.

### 3.1 Protocol Parameters

LUNA-II YF™ protocols have the following parameters:

Parameter	Range	AO-PI	FDA-PI
Dilution Factor	1-10	1.11	1.18
Min. Fluorescent Object Size (µm)	1-59	1	1
Max. Fluorescent Object Size (µm)	2-60	60	60
Green Fluorescence Exposure	1-10	7	4
Red Fluorescence Exposure	1-10	3	6

**Dilution Factor:** The dilution factor must be set properly prior to counting to ensure accuracy.

$$\text{Dilution factor} = \frac{\text{Final sample volume}^*}{\text{Yeast suspension volume}}$$

*\*final sample volume = yeast suspension volume + reagents volume*

The default dilution factor is preset as 1.11 for the AO-PI protocol and 1.18 for the FDA-PI protocol, assuming a 9:1 and 17:3 ratio of yeast suspension to reagents, respectively (see Section 4.2 Sample Preparation). Assuming a final sample volume of 20 µL, users can modify this value according to the following table:












Yeast Volume	20 µL	18 µL	17 µL	16 µL	15 µL	14 µL	12 µL	10 µL	8 µL	6 µL	4 µL	2 µL
Dilution Factor	1	1.11	1.18	1.25	1.33	1.42	1.66	2	2.5	3.33	5	10

For users handling highly dense cultures, serial dilutions and several counts with appropriately adjusted dilution factors will be necessary.

**Minimum and Maximum Fluorescent Object Size:** Fluorescent object size measures the diameter of the fluorescent signal from the nucleic acid stains. This does not correspond to the physical size of the cell. Users can adjust the parameters in 1 µm increments between 1-60 µm.

**Green and Red Fluorescence Exposure:** The exposure for each channel can be adjusted. Increase exposure if the preview image is dim and only a few cells are visible. Lower exposure if the preview image is too bright and background noise is high. Determine optimal exposure values empirically. Higher exposure levels lead to slower frame rates in the live view of cells.

Select **PROTOCOL** from the main menu.

 <b>Protocol</b>		Protocol AO-PI				
Protocol		Date 07 MAR 2016 16:00				
AO-PI		Dilution Factor (1-10)	Min. Fluorescent Object Size (1-60 $\mu$ m)	Max. Fluorescent Object Size (1-60 $\mu$ m)	Green Fluorescence Exposure (1-10)	Red Fluorescence Exposure (1-10)
FDA-PI						
		1.11	1	60	7	3
						
Load	Edit	Delete			Save as	

To create a new protocol, select one of the default protocols and press **Save as**. Using the onscreen keyboard, rename the protocol and press **Save**.

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

Protocol		Protocol AO-PI Date 07 MAR 2016 16:00				
Protocol	Dilution Factor (1-10)	Min. Fluorescent Object Size (1-60 $\mu\text{m}$ )	Max. Fluorescent Object Size (1-60 $\mu\text{m}$ )	Green Fluorescence Exposure (1-10)	Red Fluorescence Exposure (1-10)	
AO-PI						
FDA-PI						
New protocol						
	1.18	1	60	4	6	
Load	Edit	Delete	Save as			

Press **Save as**.

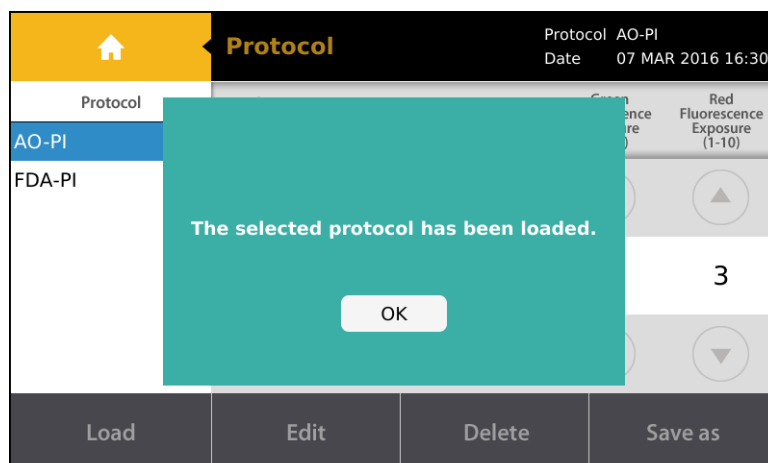
Press **Delete** to delete a selected protocol.

### 3.3 Protocol Selection

When staining cells with Acridine Orange and Propidium Iodide, select the AO-PI protocol. When staining cells with Fluorescein Diacetate and Propidium Iodide, select the FDA-PI protocol.

Select the desired protocol in the Protocol screen.

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



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

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

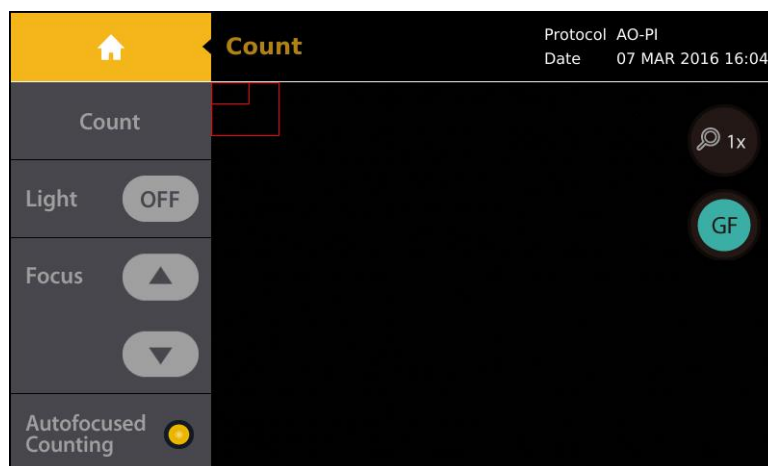


# Chapter 4 – Counting Yeast Cells

## 4.1 Instrument Preparation

Select **COUNT** from the main menu.

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



Set the desired protocol prior to counting (see Section 3.3: Protocol Selection).

## 4.2 Sample Preparation

Prepare a yeast suspension according to standard procedures. Mix gently but thoroughly to ensure that the suspension is homogenous. For yeast samples that are highly dense and/or were cultured in YPD broth, dilute the sample by at least 1:100 with Cell Dilution Buffer II prior to counting. YPD has a component that has strong esterase activity, which inhibits dye uptake into cells, leading to a weak fluorescence signal. Cell Dilution Buffer II increases cell membrane permeability, leading to increased levels of dye uptake.

- **[AO-PI]** Mix 18  $\mu$ L yeast suspension with 2  $\mu$ L Acridine Orange/Propidium Iodide Stain. Pipette gently. Incubate the sample for 10 minutes at room temperature.
- **[FDA-PI]** Mix 17  $\mu$ L yeast suspension with 1  $\mu$ L Fluorescence Signal Enhancer 1\*, 1  $\mu$ L Fluorescein Diacetate Stain and 1  $\mu$ L Propidium Iodide Stain for Yeast. Pipette gently. Incubate the sample for 10 minutes at room temperature.

*\* Fluorescence Signal Enhancer 1 is an inhibitor of ATP-dependent fluorescein efflux. When used in proper conditions, the enhancers will lower background and increase signal intensity, thereby enhancing the overall signal-to-noise ratio.*

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



Depending on sample conditions, wait for 30 seconds to 1 minute for the cells to settle within the chamber.

- ! **Important!** Prolonged incubation in Cell Dilution Buffer II can affect cell viability. Do not expose the cells to the buffer for more than 30 minutes prior to counting.

## 4.3 Slide Insertion

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

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

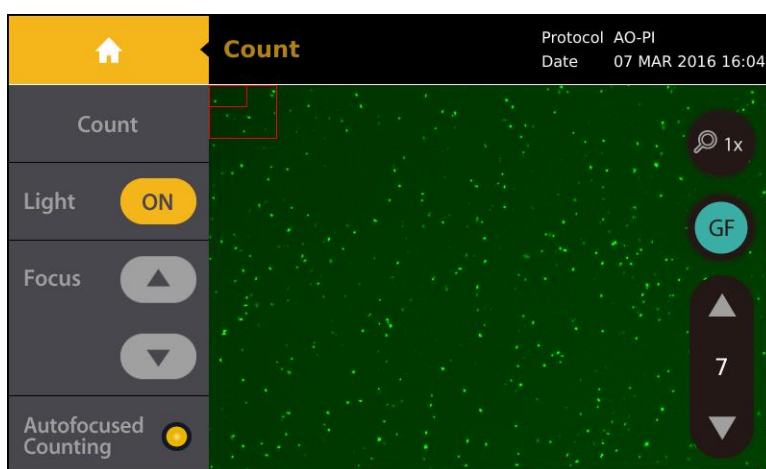
## 4.4 Color/Light Adjustment

The LUNA-II YF™ starts with the light turned off to prevent photobleaching.

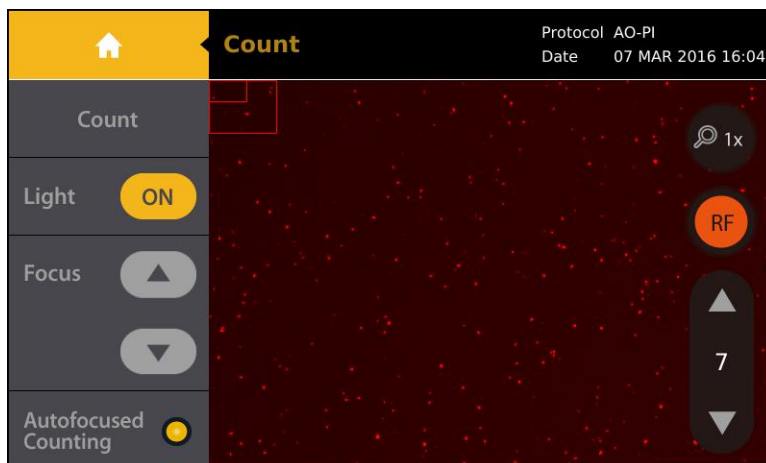
Press **[Light]** to turn on the light.

A magnifier button, fluorescence channel button, and light control bar will appear to the right of the screen.

The **GF** button indicates that the green fluorescence channel is on.



Press **GF** to change it to **RF** and switch to the red fluorescence channel.



To view the stained cells, users may adjust the intensity of each channel as needed by pressing the light control bar arrow heads (up or down).

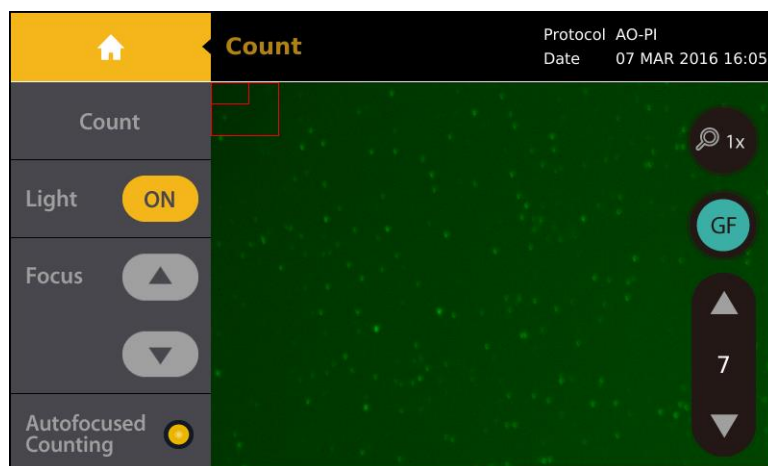
**! Important!** Adjusting the intensity levels may affect counting results. Users should only adjust intensity levels to visualize cells prior to counting. For optimal counting, maintain intensity level 7. If cell images captured during counting are too dim, adjust exposure levels in the Protocol screen.

## 4.5 Focusing

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

### 4.5.1 Autofocusing

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



### 4.5.2 Manual Focusing

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

Zoom in to the image using the magnifier button to achieve the best focus.

## 4.6 Cell Counting

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

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

Make sure the cells are not moving. If some cells are still moving, wait for all cells to settle. While waiting, turn off the light to prevent photobleaching.

Press **[Count]** to start counting. The light will turn on automatically.

The LUNA-II YF™ counts the cells in 0.7  $\mu\text{L}$ , which is comparable to seven squares on a standard hemocytometer.

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

Cell count and viability results will appear.

## 4.7 Results

The LUNA-II YF™ has onboard data analysis software that allows users to analyze data immediately.

### 4.7.1 Results: Image View

Results		
Protocol AO-PI		
Date 07 MAR 2016 16:11		
Next Count	Total cell concentration	1.7x10e6 cells/mL
Image	Live cell concentration	1.7x10e6 cells/mL
	Dead cell concentration	1.5x10e4 cells/mL
Histogram & Gating	Viability	99.1%
	Average size	4.4 um
Dilution	Total cell number	1256 cells
	Live cell number	1245 cells
Save / Print	Dead cell number	11 cells
	Dilution factor	1.11

*\*Average size indicates the fluorescent object size.*

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

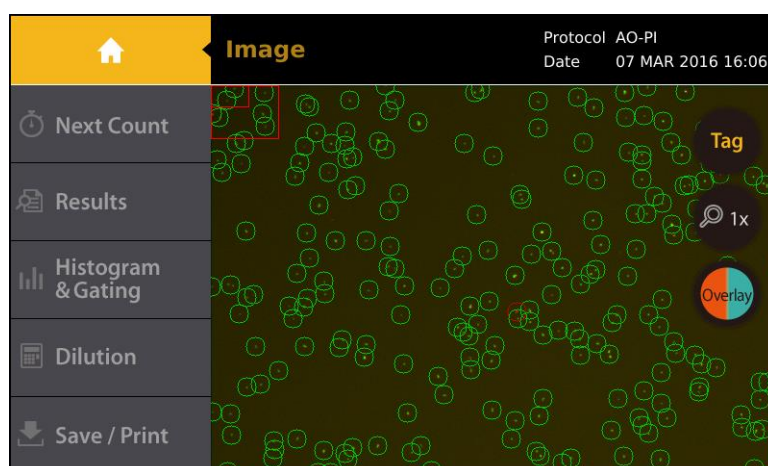
Use a finger or a stylus to navigate the image.

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

Users can view the raw images captured in each channel and a merged image of both channels. Press **Overlay** to change it to **GF** and display live cells. Press **GF** to change it to **RF** and display dead cells. Press **RF** to change it to **Overlay** and display a merged image of both live and dead cells.

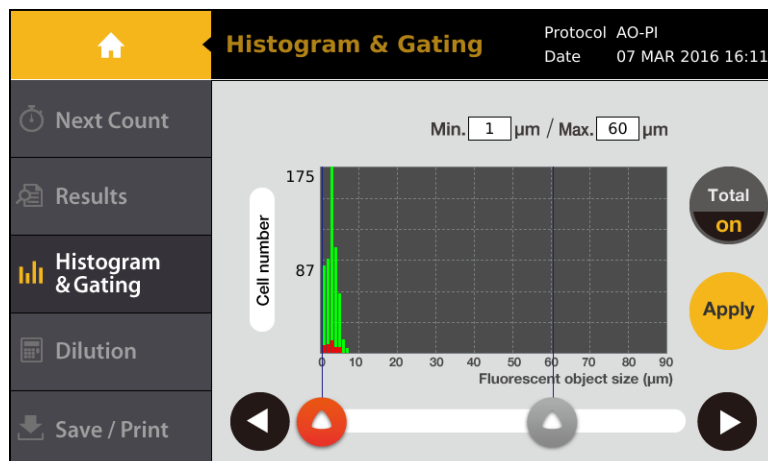
Press **Tag** in the merged image to label what was counted as live cells with green circles and dead cells with red circles. The Tag function allows users to verify the instrument's counting accuracy immediately. Press **Tag** again to remove the labels. The Tag function works for the merged image but not in the **GF** and **RF** images.

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



## 4.7.2 Results: Histogram and Gating

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



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

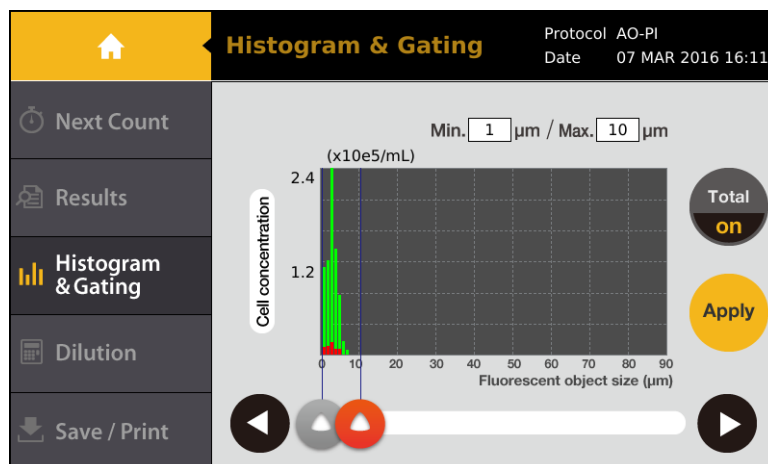
Press **Total/on** to change it to **Live/on** and display the distribution of only live cells. Press **Live/on** to change it to **Dead/on** and display the distribution of only dead cells.

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

Press the arrows on either end of the bar to alter the minimum and maximum fluorescent object size limits.

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

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



### 4.7.3 Results: Dilution Calculator

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

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

The dilution calculator starts out with the preset concentration of cells (total, live, or dead) as the current concentration. The current concentration options are **Total**, **Live**, **Dead**, and **Custom**. Users may set the concentration to be used for the dilution calculation by pressing the grey box below the Current Concentration value to be the total cell concentration, live cell concentration, dead cell concentration, or a custom cell concentration. The default concentration to be used in calculations may be set in the Settings (see Section 3.1: Protocol Parameters).

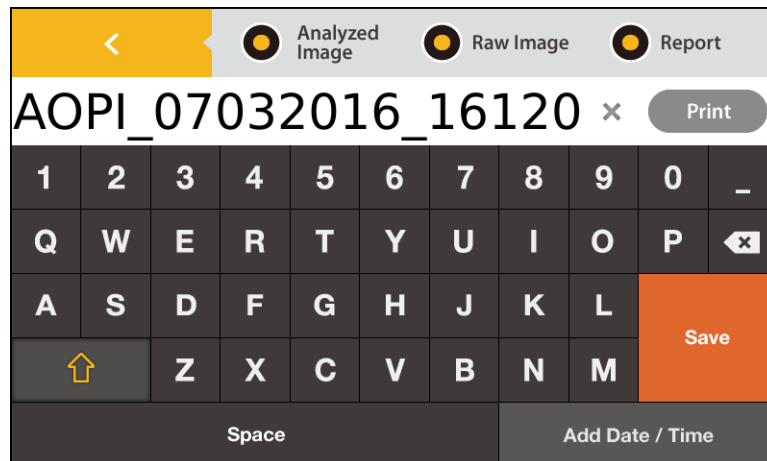
The screenshot shows the 'Dilution Calculator' interface. At the top, there is a yellow bar with a back arrow and the title 'Dilution Calculator'. Below this, the interface is split into two main columns. The left column contains three input fields: 'Current Concentration' (showing '1.7 X10e 6 /mL'), a 'TOTAL' button, 'Desired Concentration' (with empty boxes), and 'Final Volume' (with an empty box). The right column contains a numeric keypad with digits 1-9, 0, a decimal point, and a delete button, along with a yellow 'Calculate' button at the bottom.

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

Press **Calculate**.

## 4.7.4 Results: Saving and Printing

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

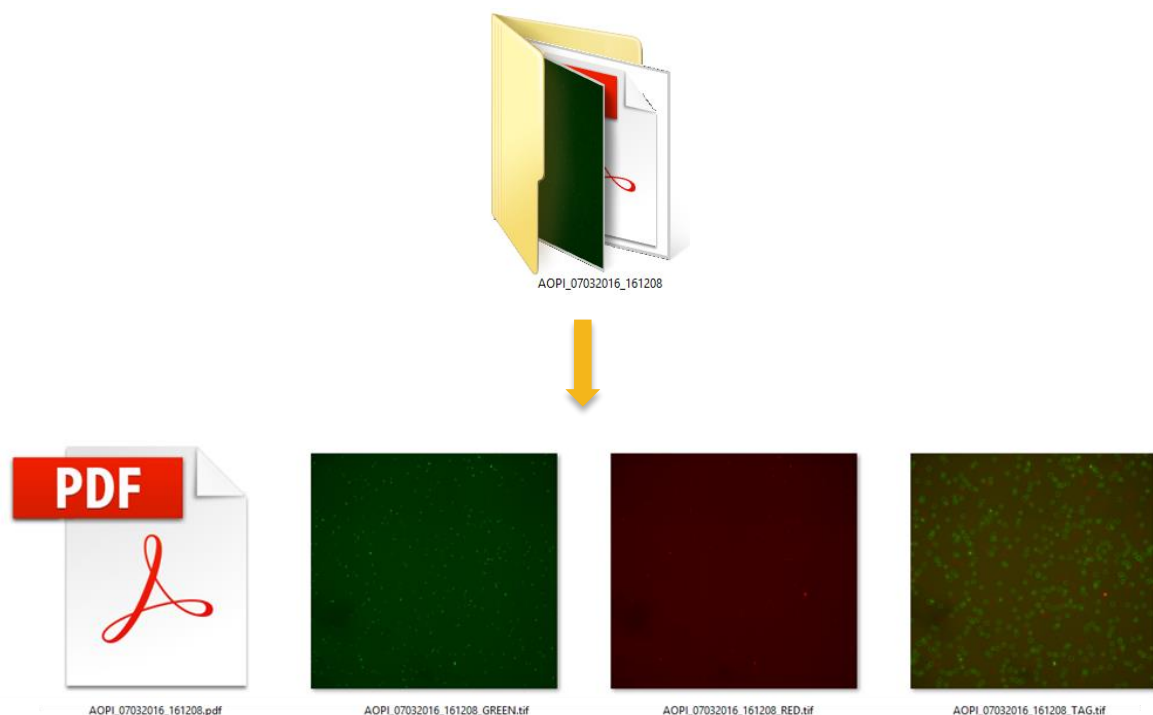


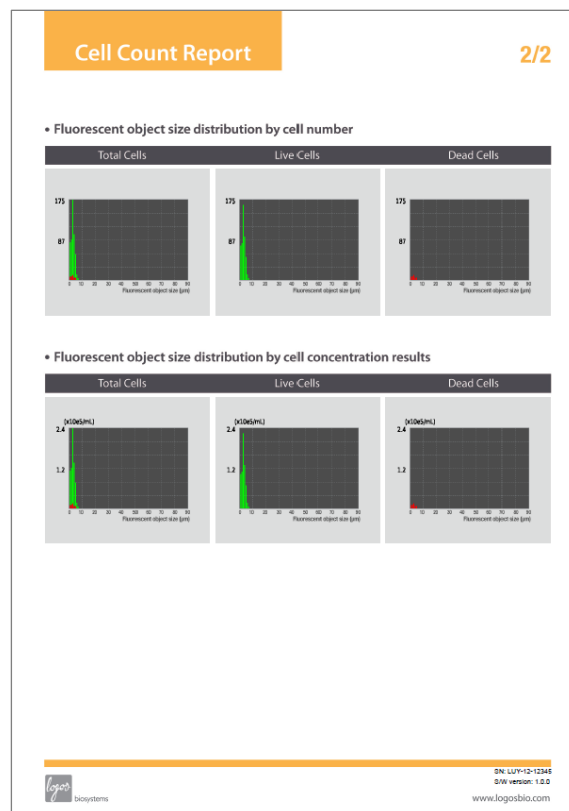
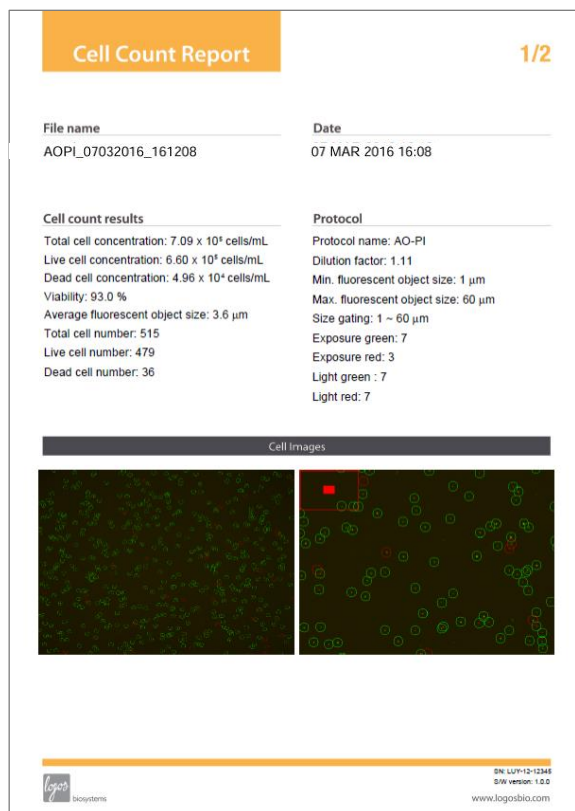
Select the desired saving options. The files to be saved as default may be set in the Settings (see Section 2.3.1: Settings: Save Options).

Using the onscreen keyboard, name the count as desired. A default file name prefix may be set in the Settings (2.3.1 Settings: Save Options).

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

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





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

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

Alternatively, press **Print** (the LUNA™ Printer is sold separately).

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

**Cell Count Report**

File name: AOPI\_07032016\_161208  
Date: 07 MAR 2016 16:08

---

**Cell count results**

[Total]:  $7.09 \times 10^5$  cells/mL  
 [Live]:  $6.60 \times 10^5$  cells/mL  
 [Dead]:  $4.96 \times 10^4$  cells/mL  
 Viability: 93.0 %  
 Avg. size: 3.6  $\mu$ m  
 Total #: 515 cells  
 Live #: 479 cells  
 Dead #: 36 cells

---

**Protocol**

Protocol name: AO-PI  
 Dilution factor: 1.11  
 Min. fluorescent object size: 1  $\mu$ m  
 Max. fluorescent object size: 60  $\mu$ m  
 Size gating: 1 ~ 60  $\mu$ m  
 Exposure green: 7  
 Exposure red: 3  
 Light green: 7  
 Light red: 7

\*Avg. size indicates the fluorescent object size.



## Chapter 5 – Review Previous Results

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

Select **REVIEW** from the main menu.

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


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


Review		Protocol	AO-PI
		Date	07 MAR 2016 16:12
Review Files	File name	Results	
	AOPI_07032016_16...	[Total cell]	7.09 x 10e5 cells/mL
		[Live cell]	6.60 x 10e5 cells/mL
		[Dead cell]	4.96 x 10e4 cells/mL
		Viability	93.0%
		Average size	3.5 um
		Total cell number	515 cells
		Live cell number	479 cells
		Dead cell number	36 cells
		Dilution factor	1.11
		G	R


\*Average size indicates the fluorescent object size.


If available in the folder, thumbnails of captured images will appear below the results. Tap on the thumbnails to make it full size. Users can view the raw images captured in each channel and a merged image of both channels. Press **Overlay** to change it to **GF** and display live cells. Press **GF** to change it to **RF** and display dead cells. Press **Tag** to view the merged image with live cells tagged with green circles and dead cells with red circles. Press the magnifier button to zoom in and out of the saved image.


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



 Review Files

 Previous Counts

 Export to USB  
(.CSV)

 Erase All

Review

Protocol

AO-PI

Date

07 MAR 2016 16:14

Name / Date	Total Cell	Live Cell	Dead Cell	Viability	Avg. Size	Protocol
AOPI_07032016_...	7.09E05	6.60E05	4.96E04	93.01%	3.55	AO-PI
07/03/2016 16:08	515	479	36			

\*Avg. size indicates the fluorescent object size.

# Chapter 6 – Maintenance and Troubleshooting

## 6.1 Turning On/Off

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

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

Turn the instrument off at the end of each day.

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

## 6.2 Cleaning

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

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

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

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

## 6.3 Troubleshooting

Problem	Possible Cause	Solution
Inaccurate cell count	Clumped cells	Gently but thoroughly pipette your cell suspension to break up aggregates prior to counting.
	Too few or too many cells	Cell concentrations of $5 \times 10^4$ - $1 \times 10^7$ cells/mL are optimal for counting. Dilute or concentrate cell suspensions accordingly.
	Fluorescence signal too strong or weak	Adjust light value and exposure.
	Visible cells uncaptured	Increase light value and exposure.
	Improper slide insertion	Make sure that the slide has been inserted into the instrument.
	Improper sample loading	Do not over- or under-fill the slide chambers. Carefully load the chambers with 10-12 $\mu$ L of cell suspension.
	Malfunction of optical components	Optical components may be dirty or damaged. Please contact your local distributor or Logos Biosystems.
	Damaged or contaminated slide	Use a new PhotonSlide™ or clean LUNA™ Reusable Slide. Wear gloves and handle by the edges to avoid smudging and contamination.
Data transfer and saving	Incorrect dilution factor	Adjust the dilution factor in the selected protocol or create a new protocol. Make sure the appropriate dilution factor has been selected.
	Poorly aligned images	Wait for cells to settle in the slide chambers prior to counting.
Background calibration taking too long	Incompatible USB drive	Some USB devices are undetectable or incompatible. Use the USB supplied with the instrument or use a USB 2.0.
	Too many files in the USB drive	Delete or transfer files.
Errors while updating software	Freezing during background calibration	If calibration takes more than 10 minutes, reset the system by turning the power off and then on again. Contact your local distributor or Logos Biosystems if calibration fails repeatedly.
	Incompatible USB drive	Some USB devices are undetectable or incompatible. Use the USB supplied with the instrument or use a USB 2.0.
	More than one software version on the USB drive	Delete previous versions of software from the USB drive before downloading new software.
	Incorrectly saved or damaged software	Use the supplied or a compatible USB drive. Download the file again into the root directory of the USB drive. Insert the USB and press [Software Updates] in the Settings screen. If the problem persists, contact your local distributor or Logos Biosystems.

## Chapter 7 – Ordering Information

	Cat #	Product	Quantity
<b>Counter</b>	L50001	LUNA-II YF™ Automated Yeast Cell Counter	1 unit
<b>Slides</b>	L12005	PhotonSlide™, 50 Slides	1 box
	L12006	PhotonSlide™, 500 Slides	10 boxes
	L12007	PhotonSlide™, 1000 Slides	20 boxes
	L12008	LUNA™ Reusable Slide	1 unit
	L12010	LUNA™ Reusable Slide Coverslips	10 units
<b>Reagents</b>	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
	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
	F23212	Cell Dilution Buffer	5 x 20 mL
	F53002	Cell Dilution Buffer II	5 x 20 mL
	F23202	Yeast Viability Kit 1	1 kit
<b>Beads</b>	F23102	LUNA™ Fluorescence Calibration Beads	2 x 1 mL
<b>Accessories</b>	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 8 - Purchaser Notification

## 8.1 Limited Use Label License: Research Use Only

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

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

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

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

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

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

## 8.2 Instrument Warranty

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

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

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

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

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

### OUT OF WARRANTY SERVICE

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



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