OLYMPUS®

INSTRUCTIONS

Cell Counter model R1

Automated Cell Counter

This instruction manual is for the Olympus Cell Counter model R1.
To ensure the safety, obtain optimum performance and to familiarize yourself fully with the use of this product, we recommend that you study this manual thoroughly before operating this product, and always keep this manual at hand when operating this product.
Retain this instruction manual in an easily accessible place near the work desk for future reference.

Optical Measuring Instrument

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You shall not inquire information relating to the source codes obtained in the above URL from Olympus.

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NOTE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Par 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 his own expense.

FCC WARNING: Changes or modifications not expressly approved by the party responsible for compliance could void the user’s authority to operate the equipment.

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In accordance with European Directive on Waste Electrical and Electronic Equipment, this symbol indicates that the product must not be disposed of as unsorted municipal waste, but should be collected separately. Refer to your local Olympus distributor in the EU for return and/or collection systems available in your country.

Table of Contents

IMPORTANT

⚠ CAUTION Safety Precautions

Before using this instrument, read this manual carefully to ensure that you know how to operate it safely and correctly. Keep this manual in an easily accessible location for future reference. The warning symbols indicate important safety related information. To protect yourself and others from personal injury or damage to property, it is essential that you read the warnings and information provided. Use the instrument as specified by Olympus. If the product is used in a manner not specified by this manual, the safety of the user may be imperiled. In addition, the product may also be damaged. Always use the equipment according to this manual.

1. Install the instrument on a sturdy and level surface. Avoid vibrations from other devices.
2. Do not touch any of the components with wet hands.
3. Operate the instrument in the conditions described in the Environmental Conditions for Operation. The temperature and humidity requirements are especially important.
4. Use the components provided or authorized by Olympus. If the proper combination of components are not used, product safety performance cannot be guaranteed.
5. Always use the AC adapter and power cord provided by Olympus. If the proper AC adapter and power cord are not used, the electric safety and EMC (Electro-Magnetic Compatibility) performance of the device cannot be guaranteed.
6. Ensure that the input voltage is compatible with the instrument's power supply voltage.
7. Connect the ground terminal of the power cord and that of the power outlet. If the device is not grounded, our intended electric safety and EMC performance of the device 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. This instrument complies with the emission and immunity requirements described in IEC61326 series.
11. Do not disassemble the instrument in any event. If the instrument is malfunctioning or broken, please contact Olympus. Disassembling the instrument invalidates its warranty.
12. The USB memory provided with this product is for exclusive use with the R1. Do not use it for any other purpose.
13. When connecting or removing the USB memory to or from a computer, be sure to follow the precautions described in the instruction manuals of the computer and its peripheral equipment.
14. When connecting the USB memory to a computer, be careful not to be infected by computer viruses.
15. Olympus is not liable for the loss or destruction of recorded data. Due to the general lifetime of data storage devices, the files saved on the USB memory may be lost after several years. As image data may be lost or destroyed unexpectedly, make frequent backups of the data. Olympus shall have no liability for any damage (including compensation for the corrupted image data) from the use or incapable use of this product. Recorded image data may be lost or destroyed in the following cases:
    a. When the user or a third party uses the USB memory incorrectly.
    b. When the user or a third party carries out a repair to the product.
    c. When USB memory is affected by static or electric noise.
    d. When the following actions are taken while the R1 is recording to USB memory or while deleting (initializing):
        - Disconnecting the USB memory.
        - Shutting down the system.
        - Pushing the power button to OFF.
        - Unplugging the AC adapter.
        - Unplugging the power cord.
        - If the instrument fails.
16. When disposing of this instrument, check and observe the regulations and rules of your local government.

Safety Symbols

The following symbols are used in this manual.

Symbol	Meaning
CAUTION	Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury.
NOTE	Indicates a potentially hazardous situation which, if not avoided, may result in damage to the instrument or other property.

The following symbols are placed on the product. Study the meaning of the symbols and always use the equipment in the safest possible manner.

Symbol	Meaning
	Indicates a non-specific general hazard. Follow the cautions given after this symbol or in the instruction manual.
	Direct Current

This system is applied with the requirements of standard IEC/EN61326-1 concerning electromagnetic compatibility.
- Emission: Class A, applied to industrial environment requirements.
- Immunity: Applied to industrial environment requirements.

Some interference may occur if this system is used in a domestic location.

CAUTION Wear proper personal protective equipment (PPE) when handling trypan blue and cell samples to avoid exposure.

CAUTION Do not reuse Cell Counting Slides. Used slides must be disposed as biohazardous waste according to the rules and regulations of your local government.

NOTE In no event shall Olympus accept any returned instrument (including its components) that might have been used or contaminated in some labs, including but not limited to, infectious disease or blood-handling labs.

Intended Use

The R1 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 R1.

1. Hold Cell Counting Slides by the edges to avoid touching the optical surface. Ensure that the optical surfaces of the slide do not become smudged, damaged, or contaminated.

2. Perform cell counting within three minutes of mixing samples with trypan blue for accurate cell viability measurements. If necessary, count your sample twice (duplicate readings) and take an average.

3. As the R1 is calibrated before shipping, recalibration before use is not necessary. However, if recalibration is needed, please refer to Section 2.3.3: Settings: Background Calibration.

Environmental Conditions for Operation

Operating Power	100 – 240 VAC, 1.2 A（AC/DC adapter)
Electrical Input	12 VDC, 2.5 A
Frequency	50/60 Hz
USB Ports	5 VDC, 500 mA
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 R1 is an image-based cell counting device that features an innovative autofocusing liquid lens and a proven counting algorithm, providing a fully automated solution for cell counting and viability analysis. Simply prepare a cell sample solution with or without trypan blue and the R1 does the rest, doing away with the subjectivity and time expenditure of manual cell counting.

The R1 counting algorithm declusters clumpy cells and counts them individually with precision. Counted cells can be gated for size and sorted into a cell cluster map to display the percentage of single cells, doublets, or triplets with a user friendly, interactive software interface.

The R1 provides:

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

The R1 automatically saves results as CSV files and provides the option to generate comprehensive PDF reports complete with the date, time, protocol used, cell images, and relevant histograms. The R1 also provides the option to review previous data.

Cell Counting Slides are disposable precision cell counting slides that have been specifically designed for the R1. The Counting Slides are manufactured with T-BOND technology to lower counting costs while maintaining the highest standard of cell counting accuracy.

1.2 Key Features

Key features	Description
Compact, space-efficient design	Lightweight and compact, the R1 maximizes space and may be used on a laboratory bench or in a biosafety cabinet.
Accuracy & precision	Sophisticated optical components and a proven counting algorithm provide accurate and reproducible results.
Autofocusing	A non-mechanical liquid lens efficiently and reliably autofocuses, removing human error and enabling accurate cell counting.
Easy-to-operate user interface	A straightforward and intuitive software allows users to capture and analyze cell count and viability data with ease.
Shortest time-to-results	With manual focusing, you are 10 seconds away from your data. With autofocusing, a mere 15 seconds.
Built-in printer	An integrated thermal printer makes record keeping effortless.
Cell size & concentration range	Cells 3-60 µm in size at concentrations ranging from 5×10^4 to 1×10^7 cells/mL are easily analyzed.
Simple dilution calculations	Onboard software calculates dilutions for users.
Onboard memory	Up to 1000 counts can be saved directly to the R1.
Customizable protocols	Up to 300 unique protocols may 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 R1 product package contains the following components.

Component	Quantity
R1	1
Power Cord (with AC adapter)	1
USB memory	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.

The following components are not included in the product package and must be purchased separately before use.

Component
Cell Counting Slides, 50 Slides (100 Counts)
Trypan blue stain, 0.4%
Printer Paper (Diameter: 32 mm, width: 58 mm)

1.4 Product Specifications

R1 Specifications	
Instrument Type	Benchtop cell counter
Dimensions (W x D x H)	195 x 237 x 272 mm
Weight	2.1 kg without the power cord and AC adapter
Cell Concentration Range	$5 \times 10^4 – 1 \times 10^7$ cells/mL
Cell Diameter Range	3-60 µm (optimal range: 8-30 µm)
Cell Viability Range	0-100%
Image Resolution	5 MP
Image Type	TIFF (Optimized for R1 only)
Software	R1 software
Documentation	PDF
Processing Time*	10** (manual focusing) or 15** (autofocusing) seconds at ~1×10^6 cell/mL
Printer Paper	Diameter: 32 mm, width: 58 mm

**Processing time may vary according to cell type and concentration.*
***This is the minimum processing time for each focusing option at the specified concentration of HeLa or HL-60 cells.*

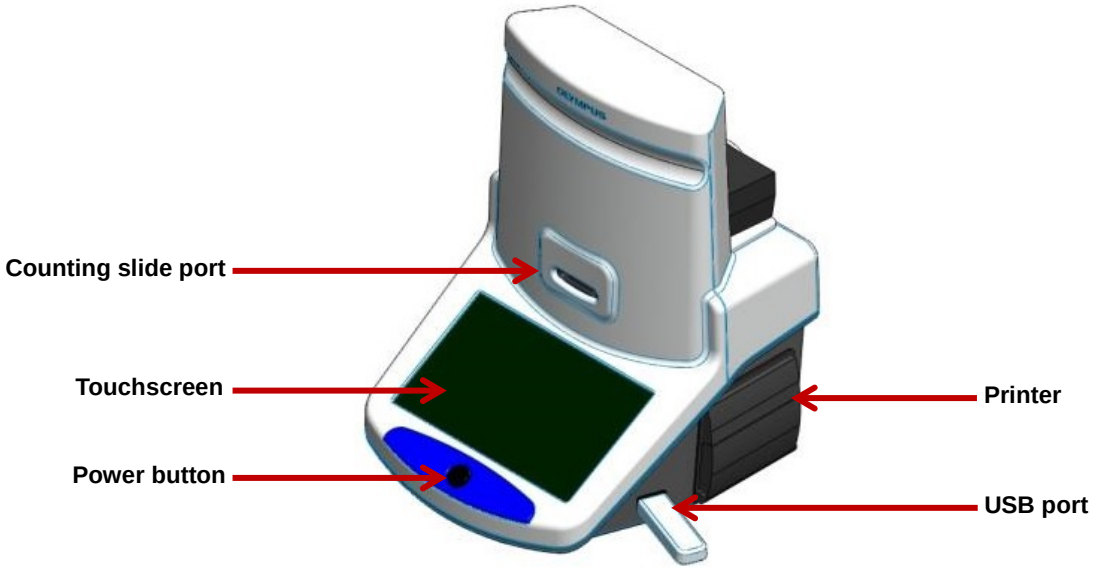
Cell Counting Slide Specifications	
Material	Polystyrene
Dimensions (W x D x H)	25 x 75 x 2.4 mm
Chamber Depth	100 µm
Chamber Volume	10 µL

R1 USB Memory Specifications	
Capacity	4 GB / 16 GB
Dimensions (W x D x H)	19.5 x 59.6 x 9.5 mm
Weight	10 g
Interface	USB 2.0
OS supported	Windows98/2000/XP/Vista/7/8/8.1
Format	FAT32

1.5 Product Description

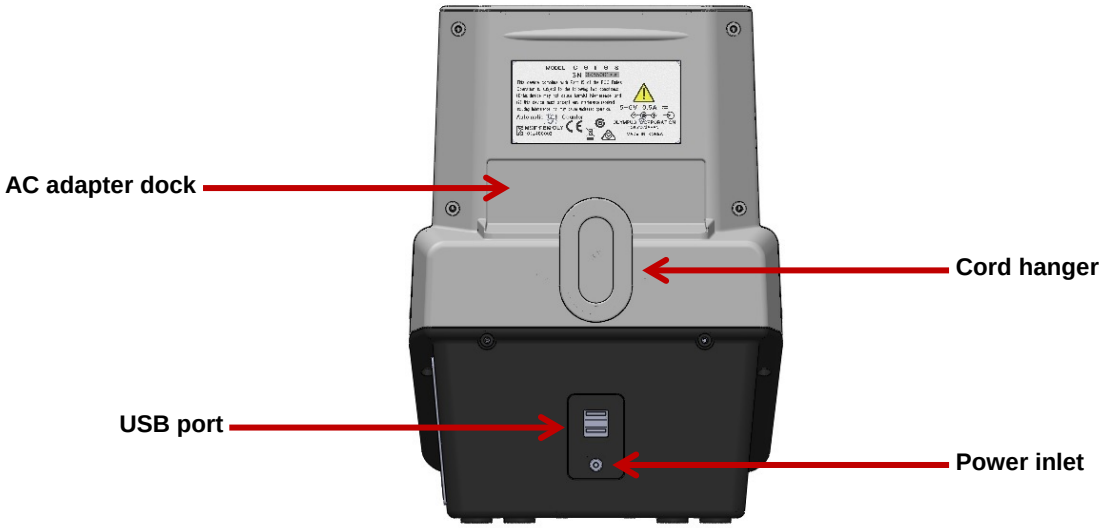
1.5.1 Front and Right Side View of the R1

The front of the R1 has a touchscreen, a power button, a counting slide port to insert Cell Counting Slides, and a USB port for easy data transfer. The right side of the R1 has a built-in printer, which allows for the immediate printing of results.



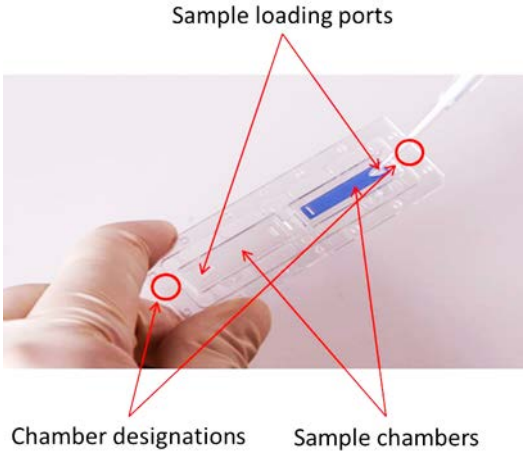
1.5.2 Rear View of the R1

The rear of the R1 has an AC adapter dock, cord hanger, and a power inlet to connect the instrument to an electrical outlet.



1.5.3 Cell Counting Slide

The Cell Counting Slide is a disposable, polystyrene cell counting slide that consists of two chambers, A and B. The depth of each chamber is 100 µm. The R1 counts the cells in 0.5 µL, which is comparable to five (1 mm x 1 mm) squares on a standard hemocytometer.



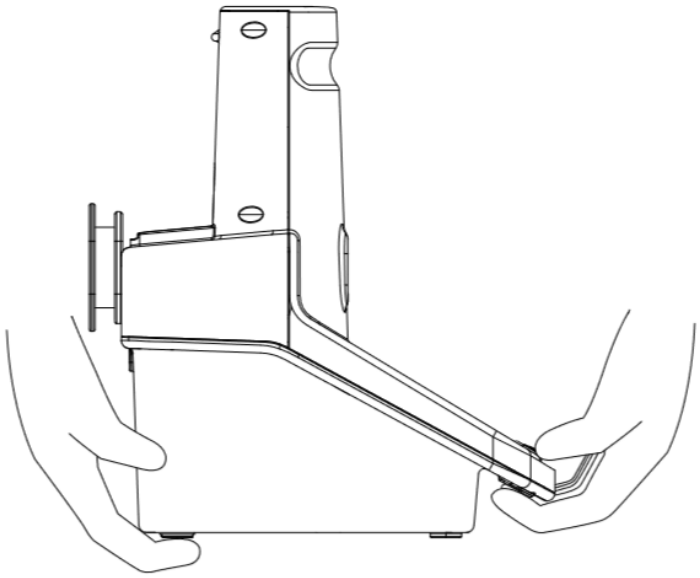
Chapter 2 – Setting up

2.1 Installation

2.1.1 Installation: Unpacking the instrument

Upon receiving the product package, unpack the instrument and its accessories to check that all parts have been included (see Section 1.3: Product Contents). Contact Olympus if anything is missing.

When transporting the instrument, carefully grip the touchscreen ledge with one hand and the bottom of the opposite side with the other hand as shown below.



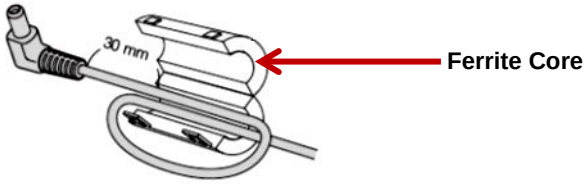
Place the R1 on a clean and level surface.

NOTE Do not hold the instrument by the top of the instrument.

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

2.1.2 Installation: Placing the ferrite core

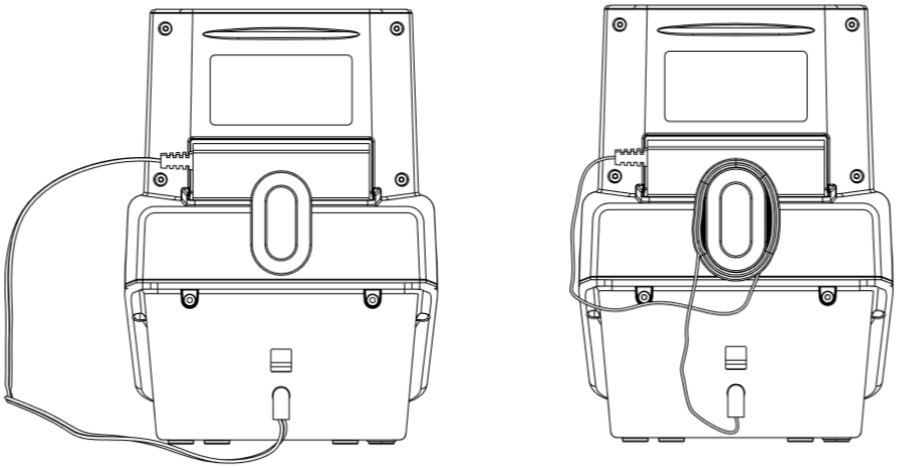
Take a ferrite core and open it. Place the plug side of the cable of the AC adapter in the ferrite core as shown in the figure. Close the ferrite core.



CAUTION The AC adapter cord and power cord are vulnerable to bending and twisting.
Do not apply excessive force.

2.1.3 Installation: Connecting the AC adapter and power cord

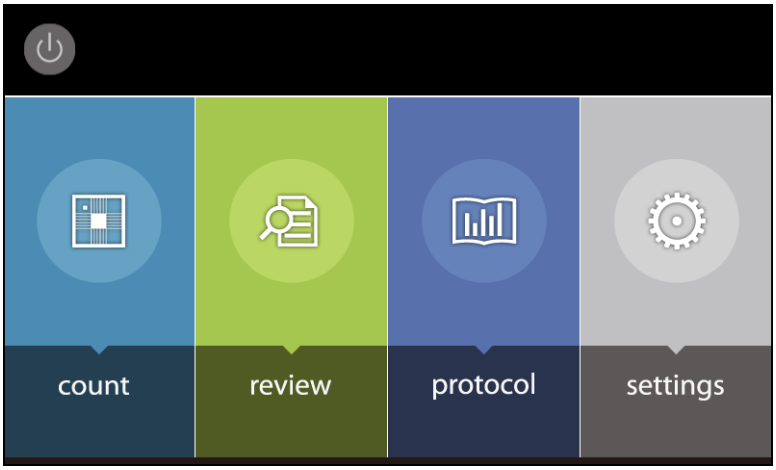
Place the AC adapter in the AC adapter dock. Wind the adapter cord neatly around the cord hanger if necessary. Insert the connector of the AC adapter into the power inlet of the instrument. Connect the power cord to the AC adapter. Connect the power cord to an electrical outlet after checking the outlet configuration in your local area.



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 main menu.

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

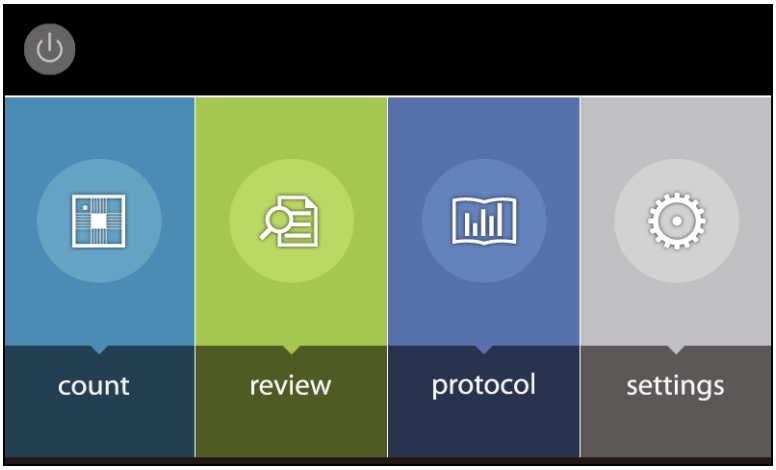


For more detailed instructions on when and how to turn the instrument on or off, see Section 6.1: Turning On/Off.

2.3 Settings

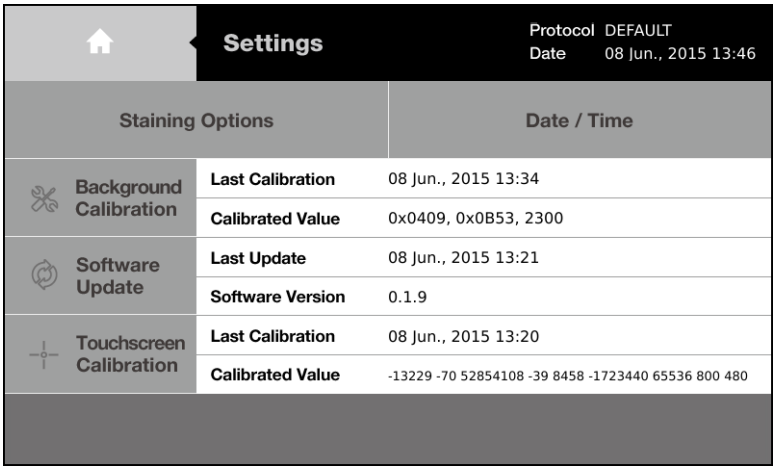
The instrument is preset at the time of manufacture and may be used immediately. Users may adjust the settings of the instrument as desired.

Select **settings** from the main menu.



The Settings screen displays:

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



Settings options:

- **2.3.1** [**Staining Options**] Select for the presence or absence of trypan blue.
- **2.3.2 [Date/Time]** Adjust the date and time of your instrument for record keeping purposes.
- **2.3.3 [Background Calibration]** Perform background calibrations with each software update.
- **2.3.4 [Software Updates]** Update Software to the most recent version.
- **2.3.5 [Touchscreen Calibration]** Calibrate the touchscreen.

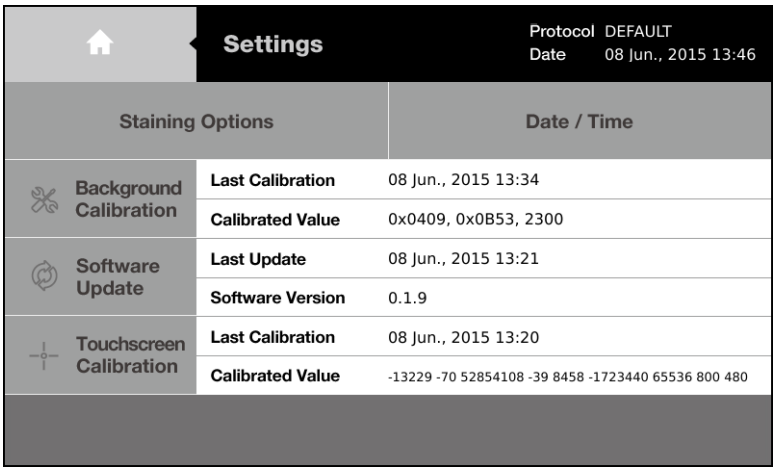
2.3.1 Settings: Staining Options

Users can select for the use or absence of trypan blue for cell counting with the R1.

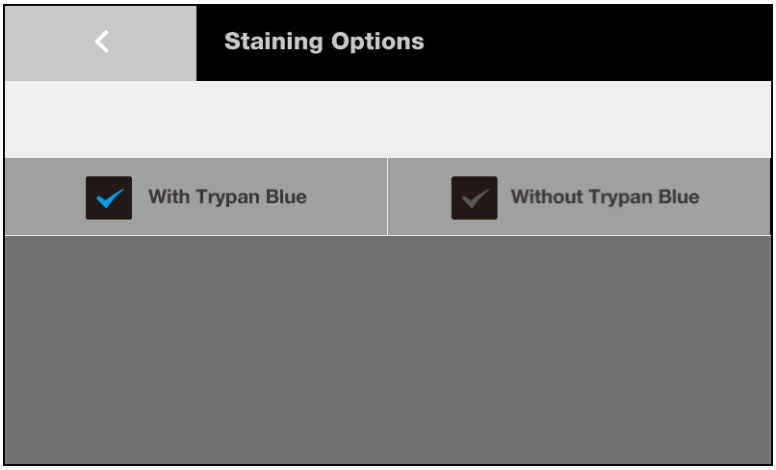
Option	Description
With Trypan Blue*	This option is used when cell samples are mixed 1:1 with 0.4% trypan blue for regular bright field counting. This option generates cell viability data. Ensure that the dilution factor in your set protocol is set to “2”. **
Without Trypan Blue	When samples are not mixed with trypan blue, turn on this option and follow the directions in the message boxes. Ensure that the dilution factor in your set protocol is set to “1”. **

*The R1 is optimized for use with trypan blue. Low contrast from not using trypan blue may lead to suboptimal results.
**The dilution factor will not change automatically. Upon changing the Staining Options, users must manually adjust the dilution factor accordingly. Failure to adjust the dilution factor will lead to an inaccurate calculation of cell concentrations.

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

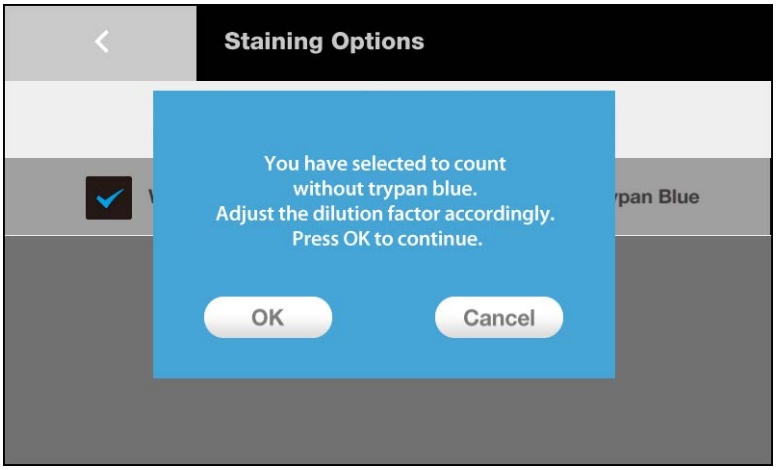


The selected option will be marked with a blue ✔.

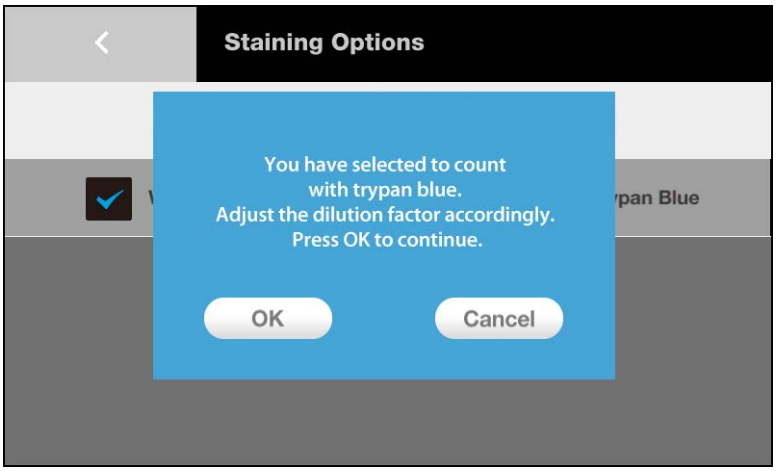


Change the staining option by pressing the unselected option.

When **Without Trypan Blue** is selected, the following window will appear.



When **With Trypan Blue** is selected, the following window will appear.



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

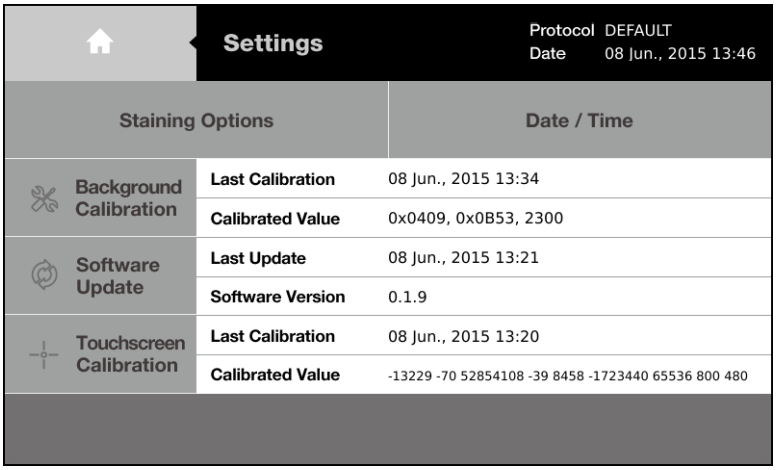
Press **<** to return to the Settings screen.

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

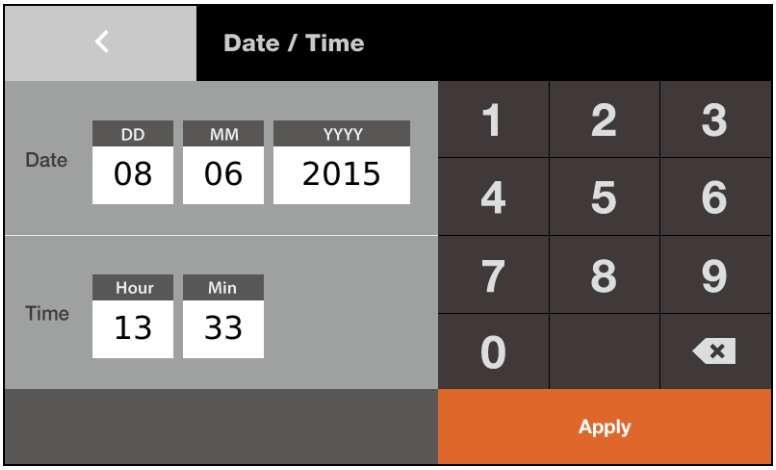
2.3.2 Settings: Date/Time

The R1 uses a 24-hour clock and is preset to Korean time. Adjust the settings to the local date and time for accurate record keeping.

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



Select the desired field to delete the existing value. Input the desired values with the number panel on the right.



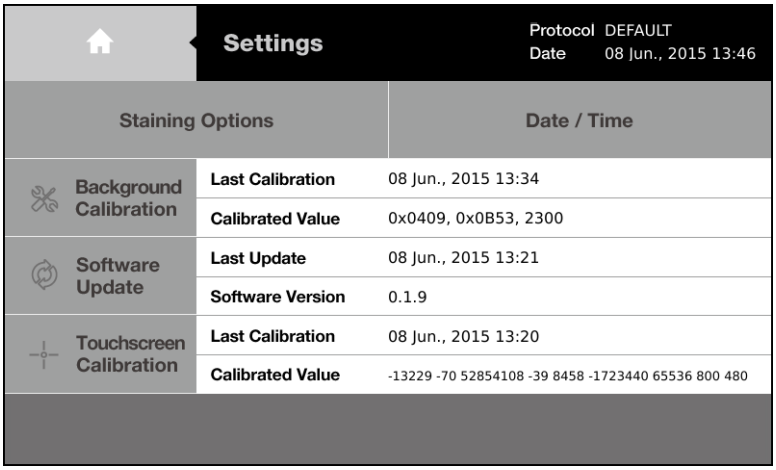
Press **Apply** to save changes.

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

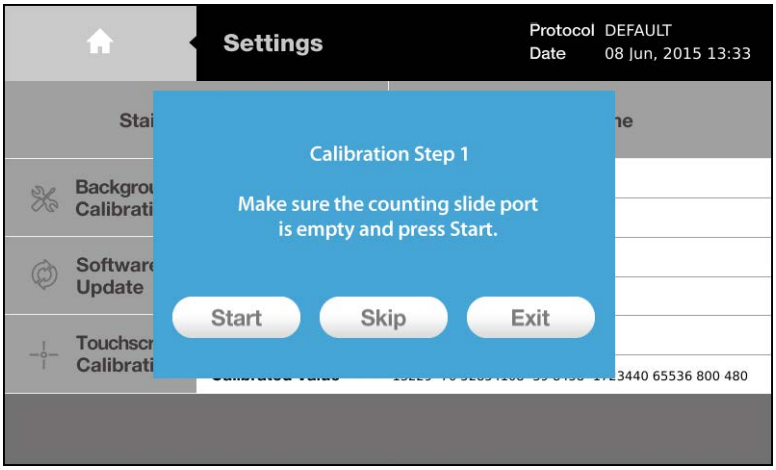
2.3.3 Settings: Background Calibration

Background calibration adjusts for the specific shade of the trypan blue stain used for counting and is a prerequisite for the successful detection of cells. Users must recalibrate the background after each software update or when using a different brand or concentration of trypan blue.

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



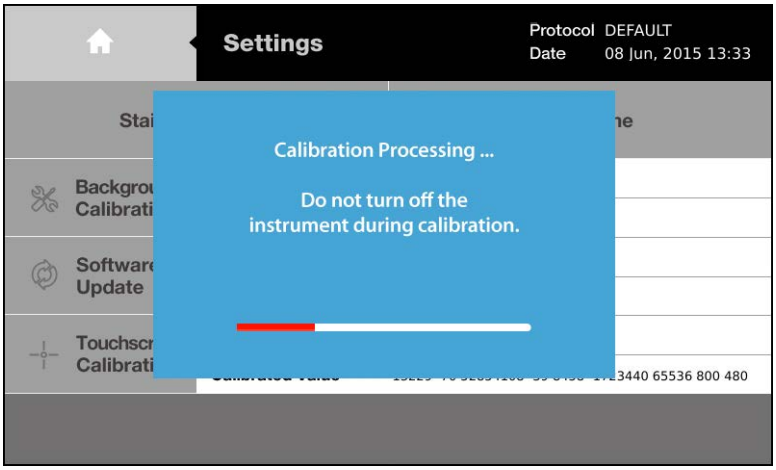
A window will appear with directions for Calibration Step 1.



The counting slide port should be empty for Calibration Step1. If there is a slide in the counting slide port, remove it from the instrument.

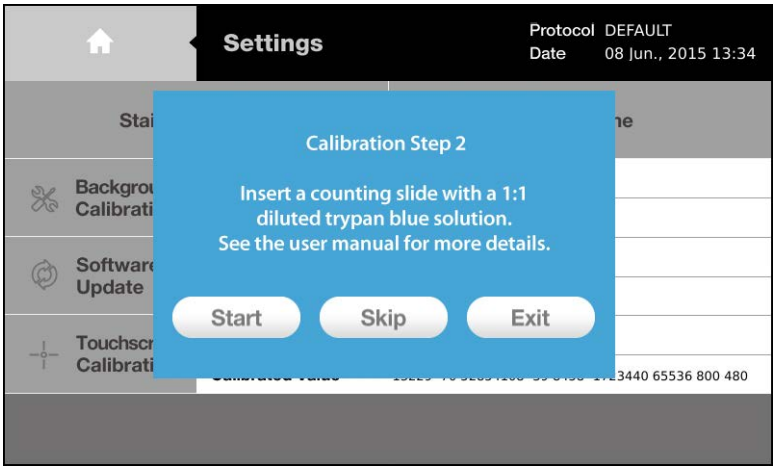
Press **Start**.

Do not turn the instrument off during this process.



Mix one part 0.4% trypan blue stain with an equal volume of distilled water, PBS, or plain medium. Put 10 µL of the diluted trypan blue stain into the chamber of a new Counting Slide.

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

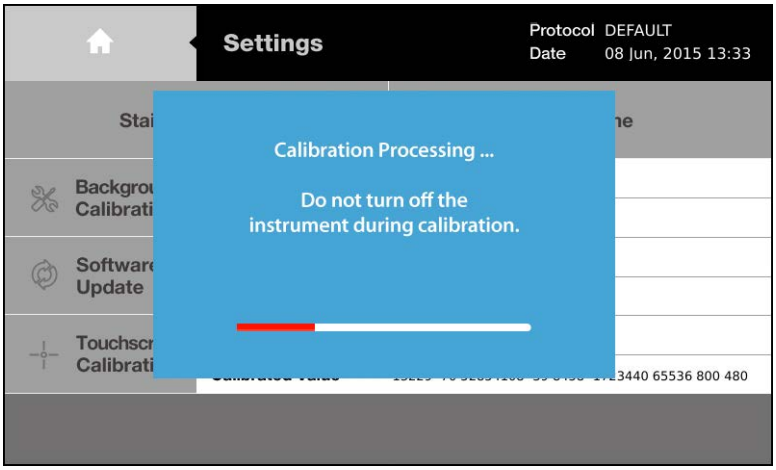


Insert the Counting Slide face up and sample-side first into the counting slide port.

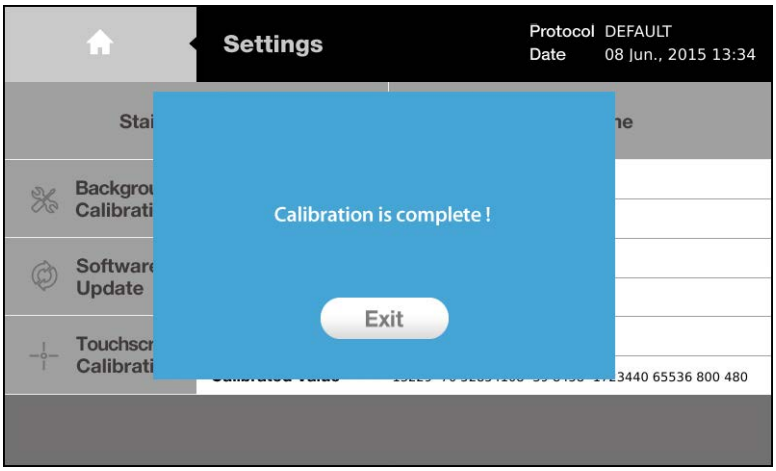
NOTE Do not insert the Counting Slide facedown.

Press **Start**.

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



Press **Exit** to return to the Settings screen when Calibration Step 2 is complete.



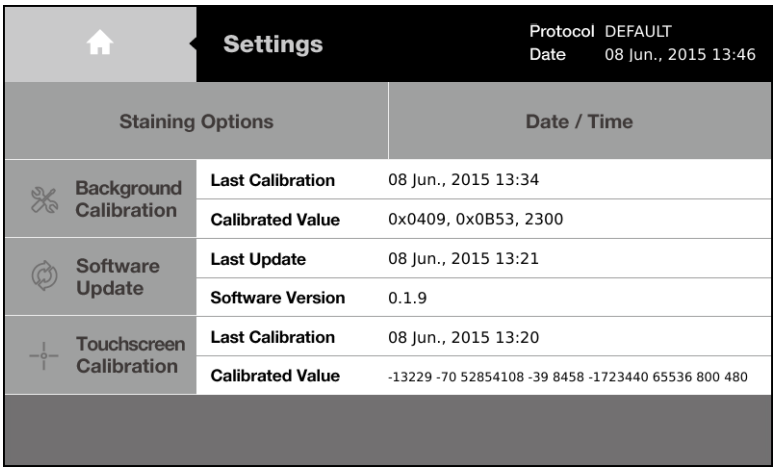
The background calibration value and date will have changed in the Settings Screen.

2.3.4 Settings: Software Updates

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

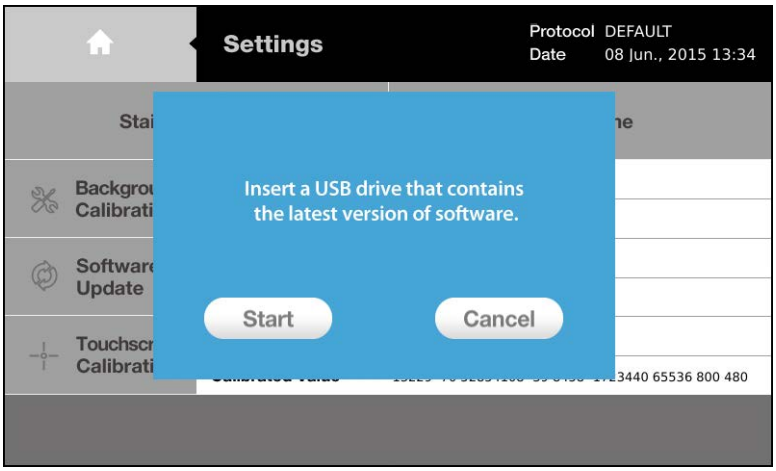
Download the most recent version from the Olympus website (www.olympus-lifescience.com) into the root directory of a compatible USB memory.

Press **[Software Updates]** in the Settings screen.



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

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



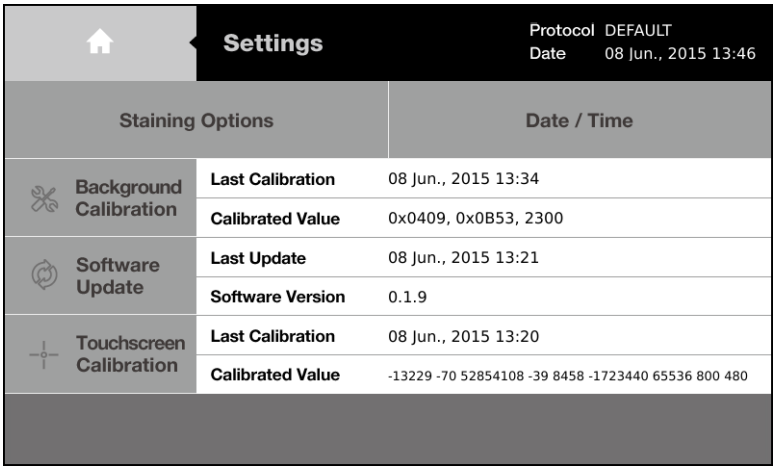
The date and version of the last software update will change automatically in the Settings screen.

! **Important!** Users must recalibrate the background after each software update (see Section 2.3.3: Settings: Background Calibration).

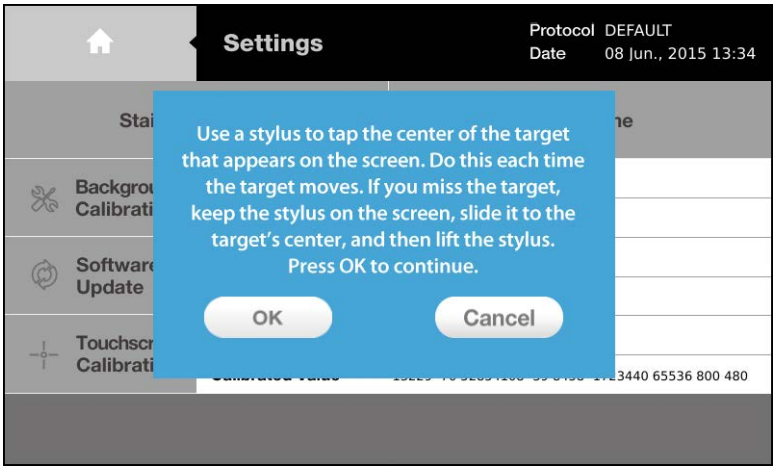
2.3.5 Settings: Touchscreen Calibration

Calibrate the touchscreen when the response of the touchscreen is slow or inconsistent. Calibration must be done with a stylus.

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



A window will appear with instructions on how to calibrate the touchscreen. Press **OK** to continue.



A small target will appear in the top left corner of a blank screen. Use a stylus to tap the center of the target. Do this each time the box moves. If you miss the target, keep the stylus on the screen, slide it to the target's center, and then lift the stylus.

The Settings screen will return when calibration is complete.

The touchscreen calibration value and date will have changed in the Settings screen.

Chapter 3 – Protocol Settings

The R1 provides a default protocol that can be used for most common cell lines. Users may create and save up to 300 unique protocols.

3.1 Protocol Parameters

The R1 protocols have the following modifiable parameters:

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

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

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

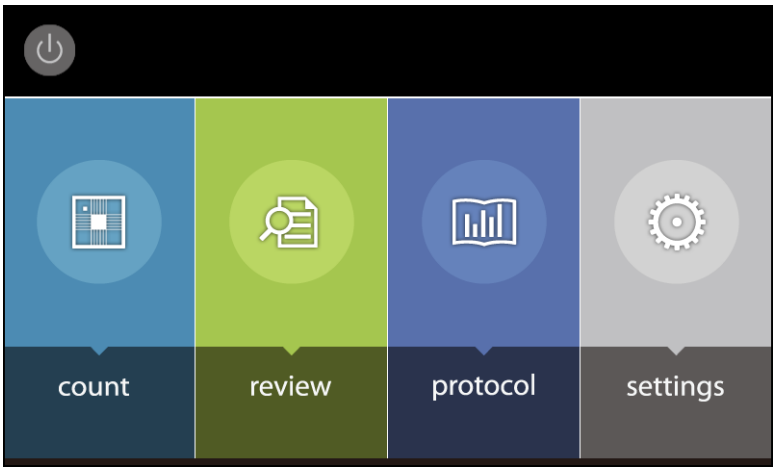
Roundness: As not all cells are completely spherical, adjusting the roundness allows for the detection of a variety of cells. Higher percentages lead to the counting of rounder cells and excludes objects with less roundness. Lower percentages are suitable for counting cells with irregular shapes.

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

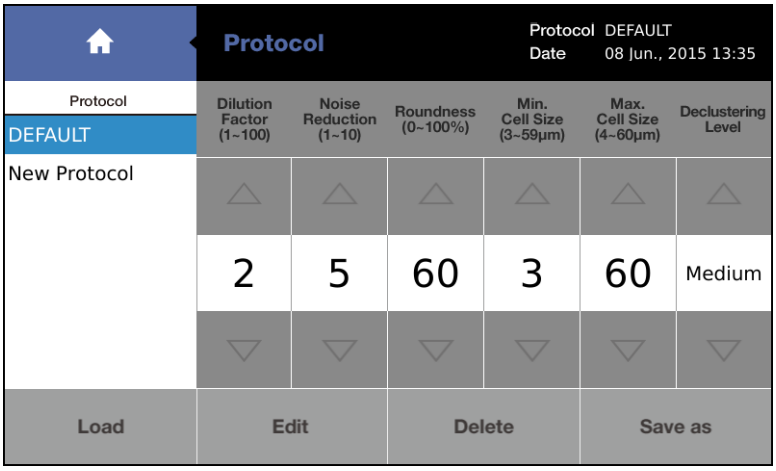
Declustering Level: The declustering function allows for the efficient detection of a variety of cells that may clump or grow in clusters. Higher levels of declustering will increase counting time. This function is helpful for counting sticky cells or rod-shaped spores.

3.2 Creating and Editing Protocols

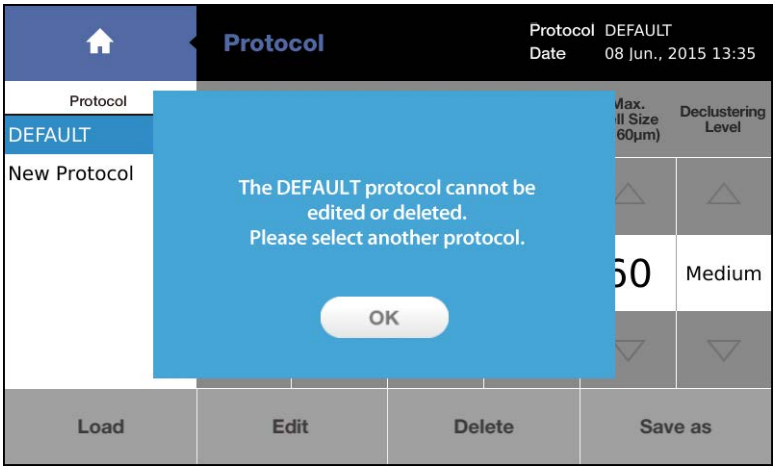
Select **protocol** from the main menu.



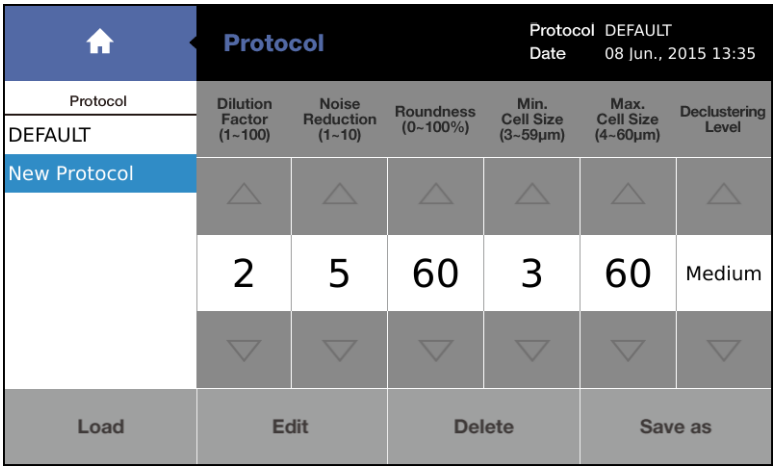
The Protocol screen includes a list of saved protocols. The selected protocol is highlighted in blue. The parameters of the selected protocol are displayed in the right panel.



The DEFAULT protocol cannot be modified or deleted.

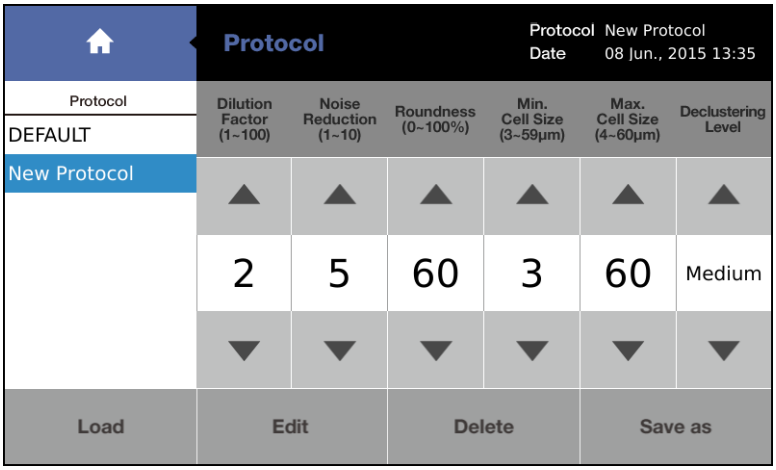


To create a new protocol, select **New Protocol** and press **Load**.

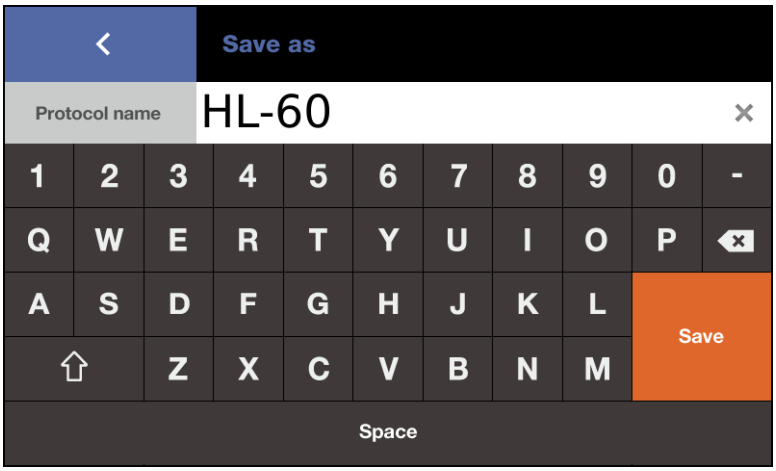


Press **Delete** to delete the selected protocol.

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



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

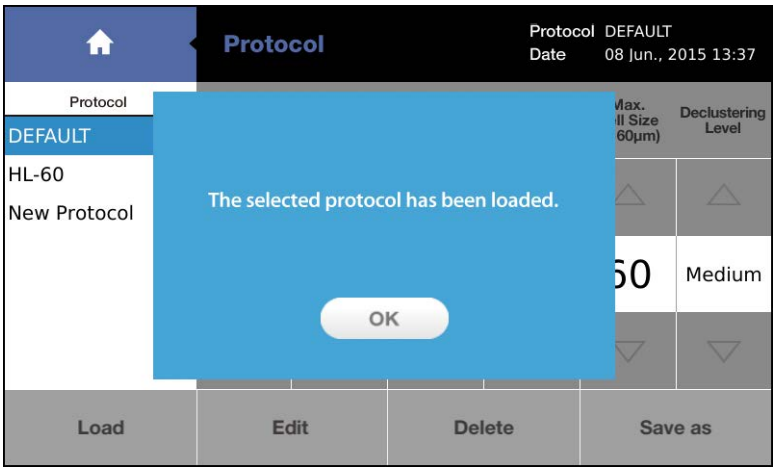


The newly created protocol will appear in the list of protocols in the Protocol screen.

3.3 Protocol Selection

Select the desired protocol in the Protocol screen.

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



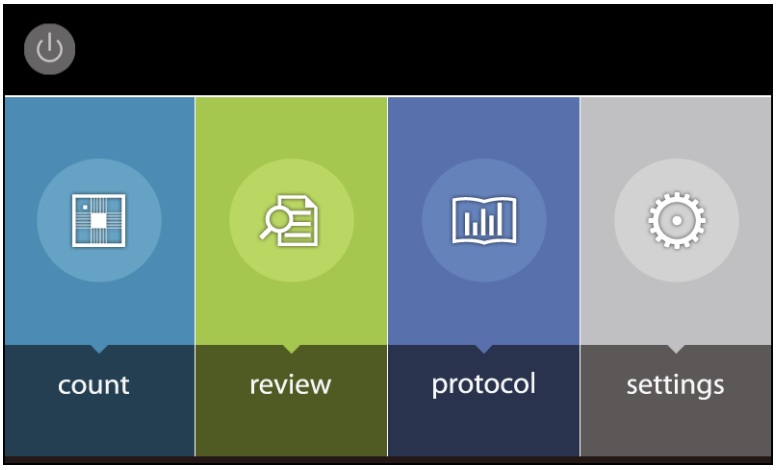
Now the instrument is ready to count cells with the selected protocol.

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

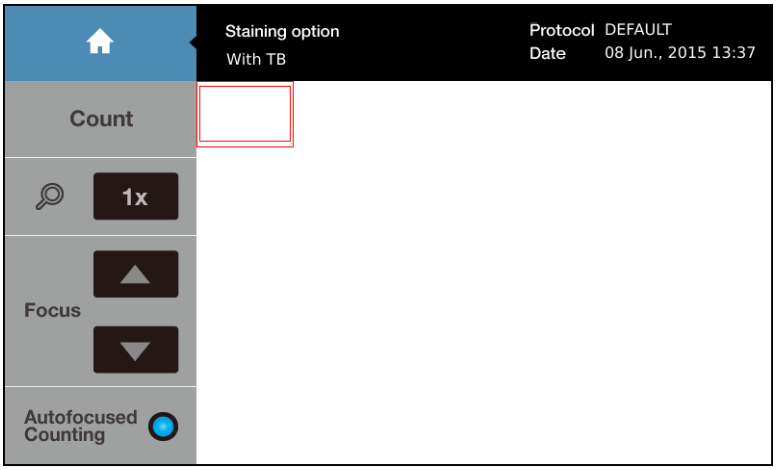
Chapter 4 – Counting Cells

4.1 Instrument Preparation

Select **count** from the main menu.



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



To change the staining option, see Section 2.3.1: Settings: Staining Options.

To change the protocol, see Section 3.3: Protocol Selection.

To change the date and time, see Section 2.3.2: Settings: Date/Time.

4.2 Sample Preparation

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

Mix 10 µL of the cell suspension with 10 µL of trypan blue stain. Pipette gently.

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

4.3 Slide Insertion

Insert the Counting Slide face up and sample-side first into the counting slide port of R1. The R1 can only analyze the inserted chamber.

NOTE Do not insert the Counting Slide facedown.

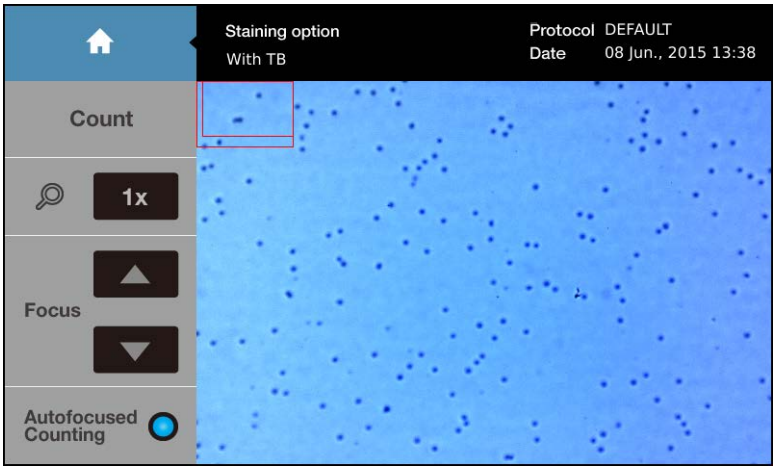
A live image of the cells will appear on the screen. If an image does not appear, the cell counting slide may be inserted incorrectly.

4.4 Focusing

The R1 provides two focusing options: autofocusing and manual focusing. The R1 has an autofocusing algorithm optimized for cell counting that works in tandem with a novel focusing mechanism that rapidly obtains the Z position of the sample by the application of a small voltage to a liquid lens. The elimination of mechanical parts removes noise and significantly reduces the need for servicing.

4.4.1 Autofocusing

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



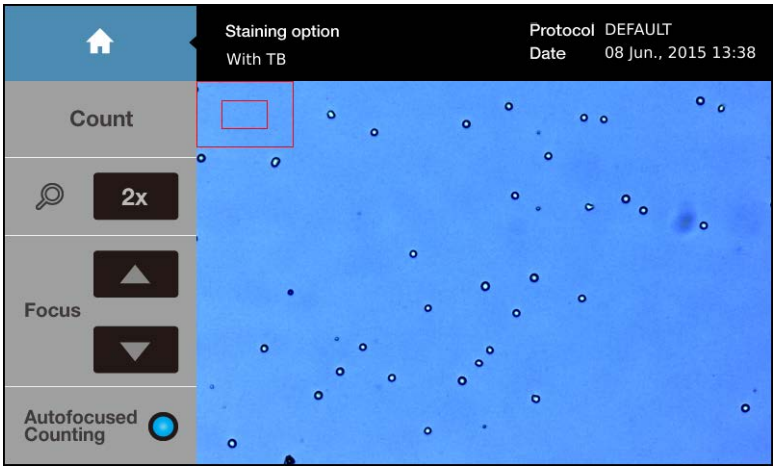
4.4.2 Manual Focusing

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

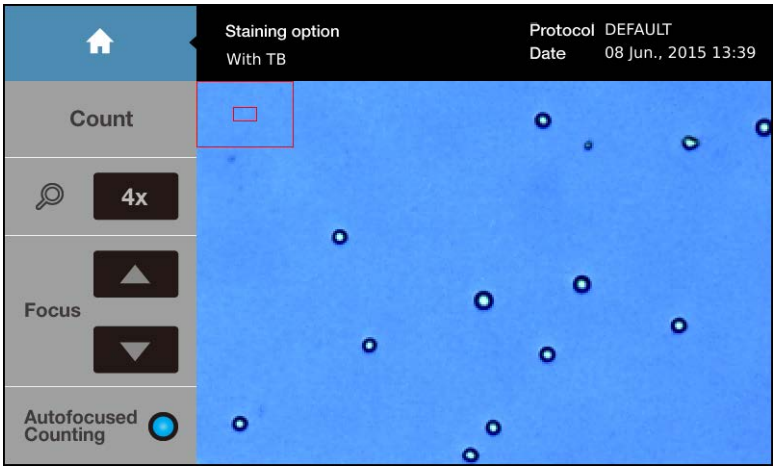
4.5 Cell Counting

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

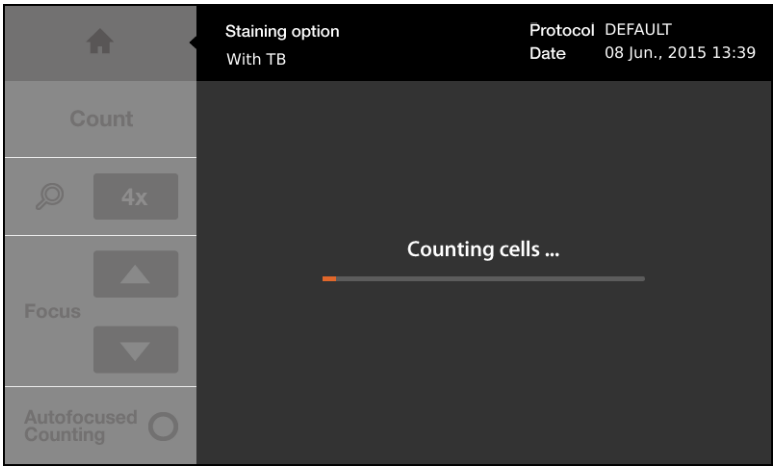
Press the magnifier button to zoom in and out of the image.



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

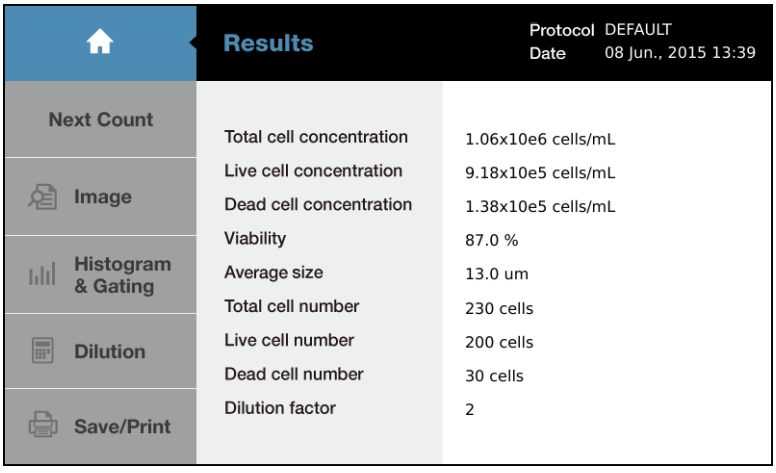


The R1 counts the cells in 0.5 µL, which is comparable to five (1 mm x 1 mm) squares on a standard hemocytometer.



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

Cell count and viability results will appear.

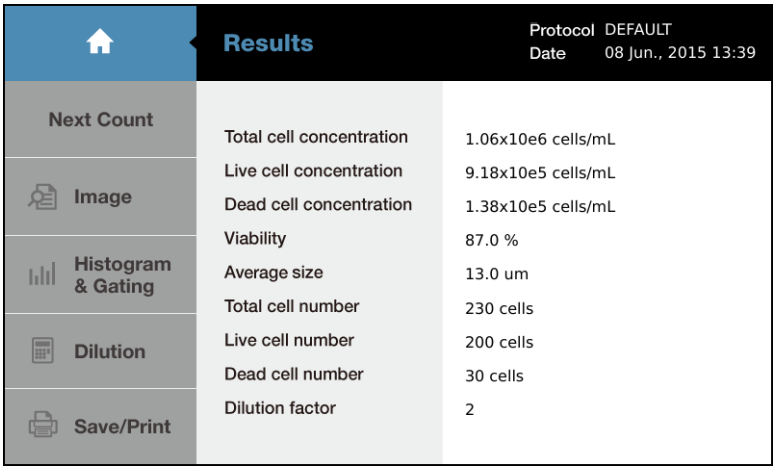


4.6 Results

The R1 has onboard data analysis software that allows users to analyze cell count and viability data immediately.

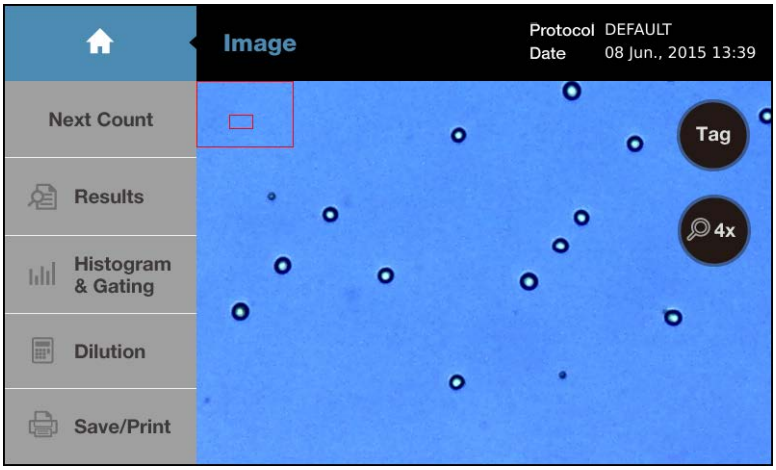
4.6.1 Results: Image View

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



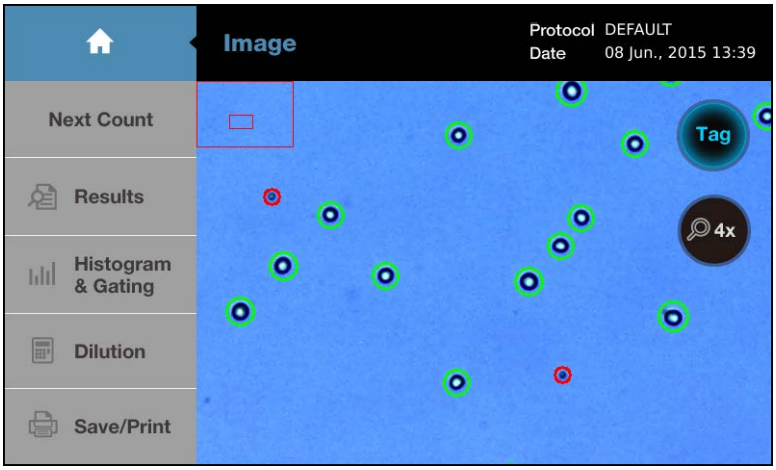
Use a finger or a stylus to navigate the image. The **Tag** and magnifier buttons are to the right of the image.

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



The Tag function allows users to verify the instrument's counting accuracy immediately.

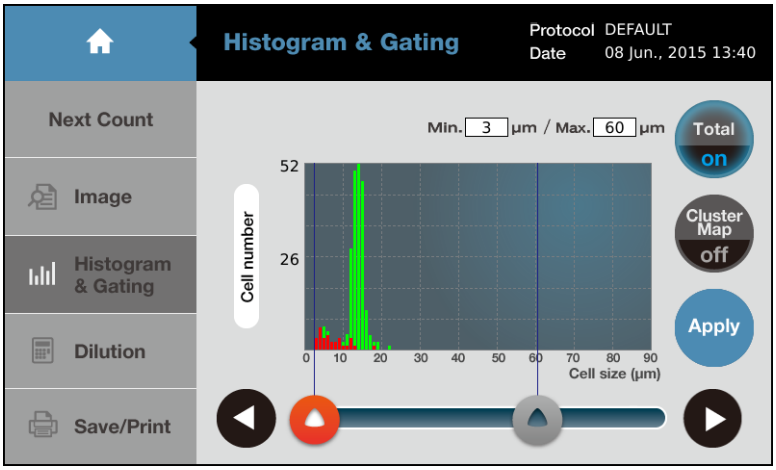
Press **Tag** to label what was counted as live cells with green circles and dead cells with red circles.



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

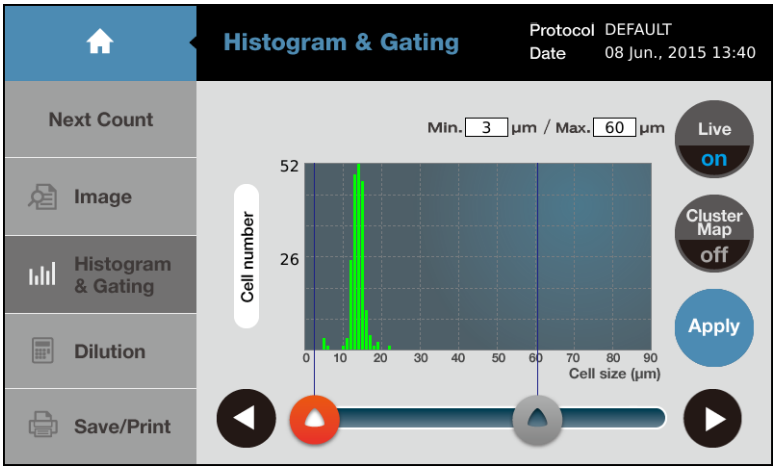
4.6.2 Results: Histogram and Gating

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

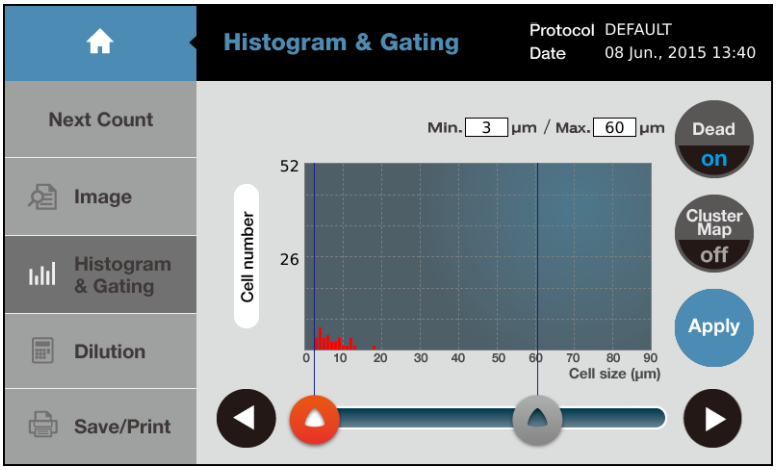


Users can review the distribution of cells according to their sizes. Green bars represent live cells and red bars represent dead cells. The **Total/on** button indicates that live and dead cells are both represented.

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

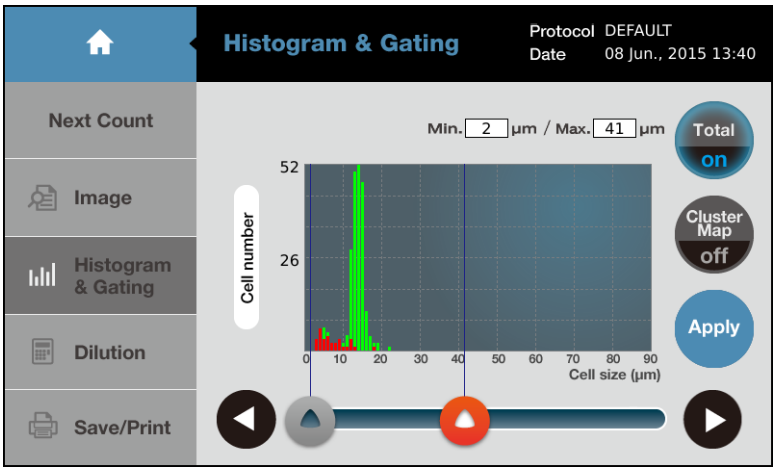


Press **Live/on** to change it to **Dead/on** and display the size distribution of only dead cells.



The R1 provides a gating function that can be controlled by the gating bar on the bottom of the screen. Select the desired light grey limit icon. The selected icon will become red.

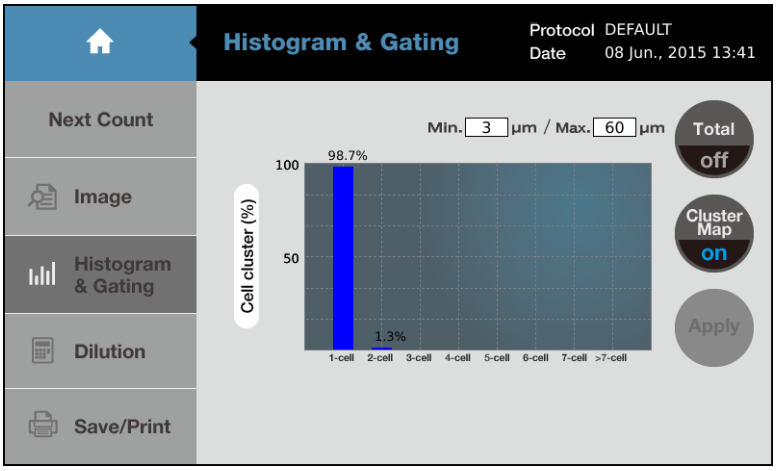
Press the arrows on either end of the size to alter the minimum and maximum size limits. The gating function is helpful for monitoring co-cultured cells with distinct sizes and the exclusion of noncellular particles.



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

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

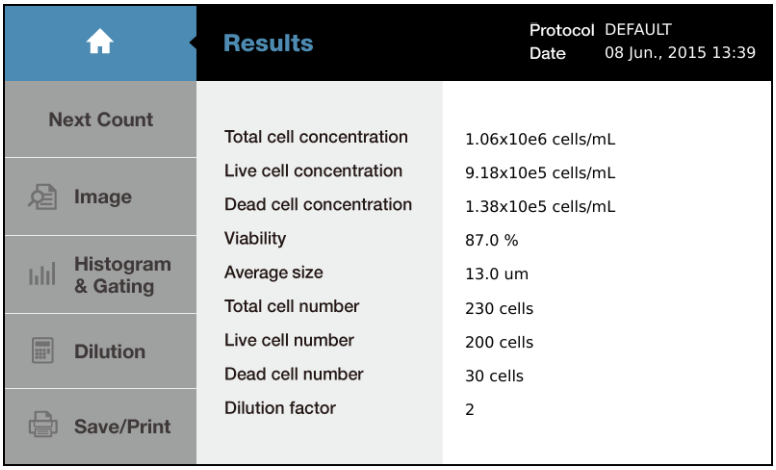
Press **Cluster Map/off** to change it to **Cluster Map/on** and show the distribution of cell clusters.



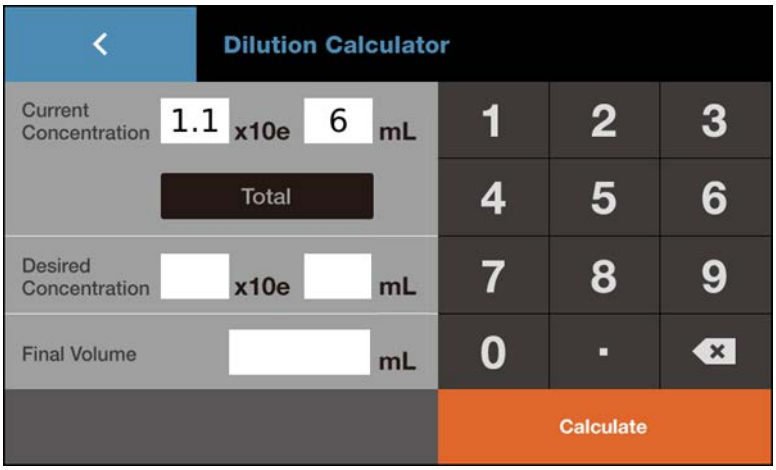
4.6.3 Results: Dilution Calculator

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

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



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



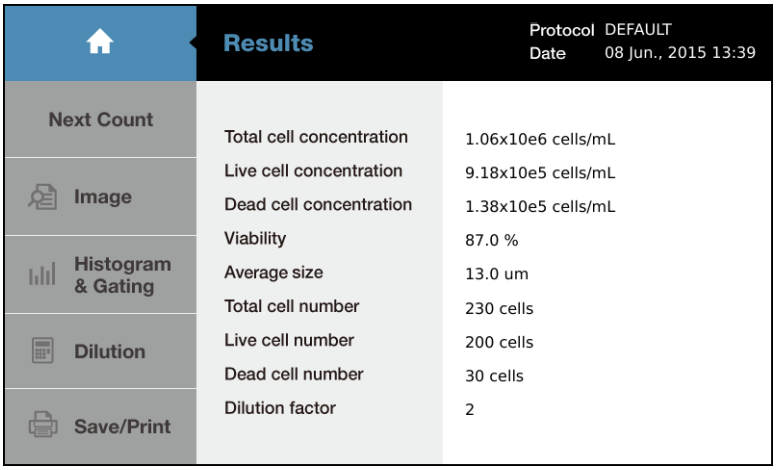
Input the values into the blanks for the desired final concentration and volume.

Press **Calculate**.

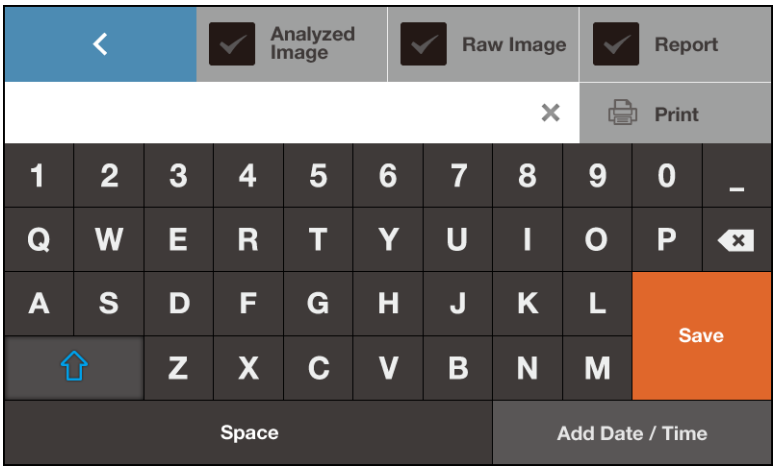
4.6.4 Results: Saving and Printing

The R1 provides the option of saving and/or printing results.

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



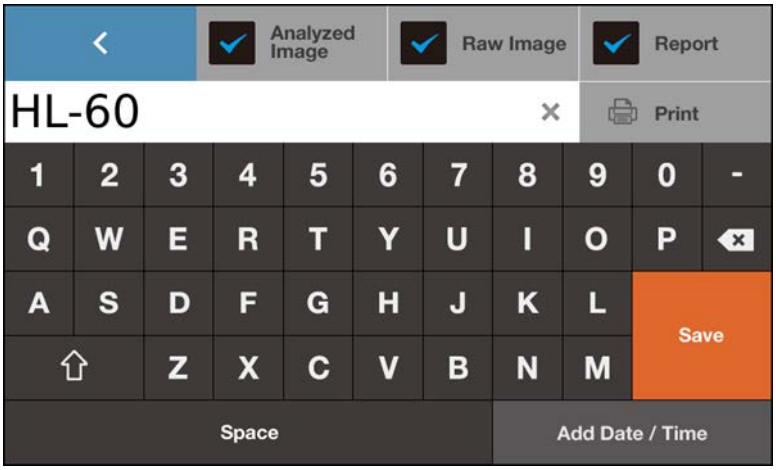
The Save/Print screen has three saving options.



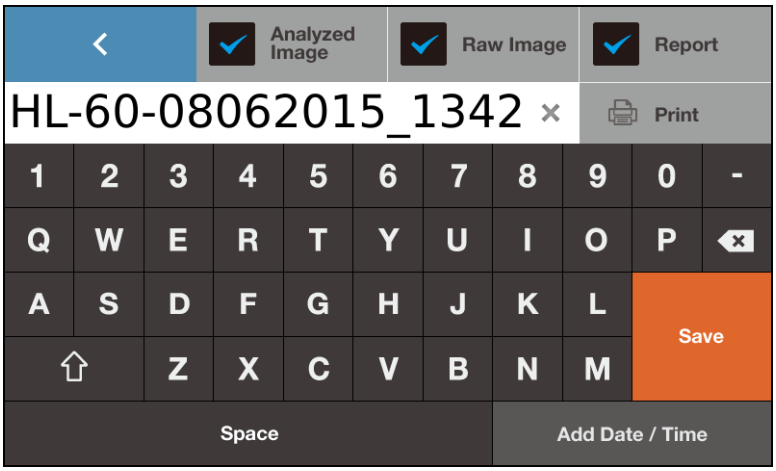
Saving Options		Description
Analyzed Image		Tagged image of live and dead cells
Raw Image		Untagged image of cells
Report		PDF report with count data and histograms

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

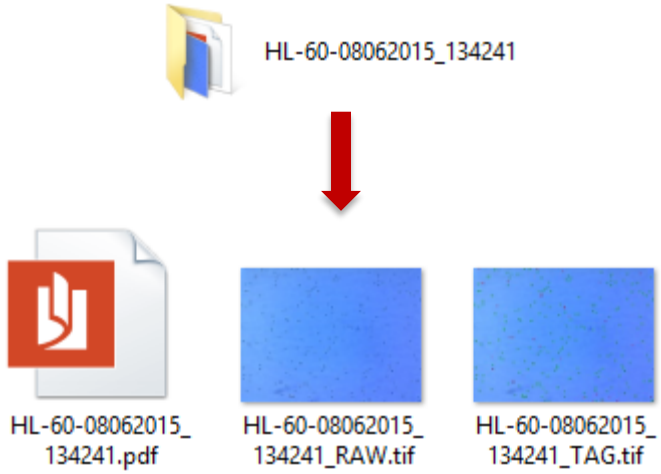
Using the onscreen keyboard, name the count as desired.



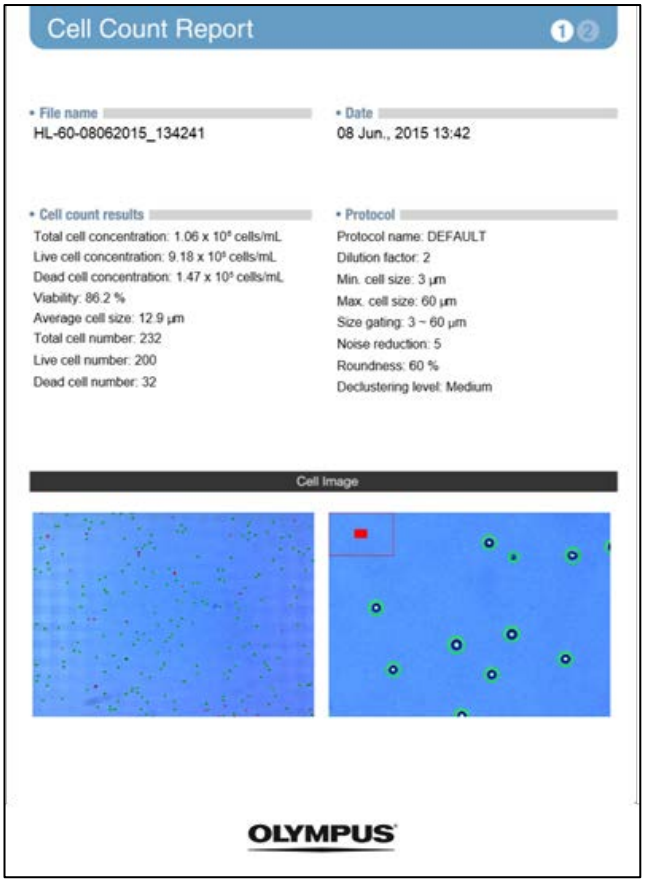
Users may add the date to the name by pressing the **Add Date/Time** button.



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



HL-60-08062015_134241.pdf



Cell Count Report 1 2

• File name
HL-60-08062015_134241

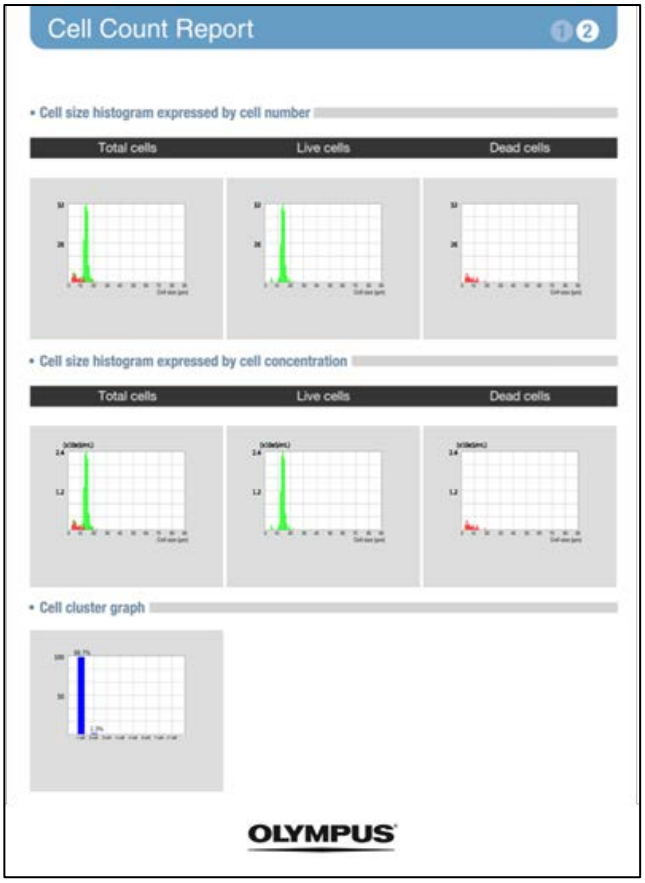
• Date
08 Jun., 2015 13:42

• Cell count results
Total cell concentration: 1.06 x 10^6 cells/mL
Live cell concentration: 9.18 x 10^5 cells/mL
Dead cell concentration: 1.47 x 10^5 cells/mL
Viability: 86.2 %
Average cell size: 12.9 µm
Total cell number: 232
Live cell number: 200
Dead cell number: 32

• Protocol
Protocol name: DEFAULT
Dilution factor: 2
Min. cell size: 3 µm
Max. cell size: 60 µm
Size gating: 3 ~ 60 µm
Noise reduction: 5
Roundness: 60 %
Declustering level: Medium

Cell Image

OLYMPUS



Cell Count Report 1 2

• Cell size histogram expressed by cell number

Total cells | Live cells | Dead cells

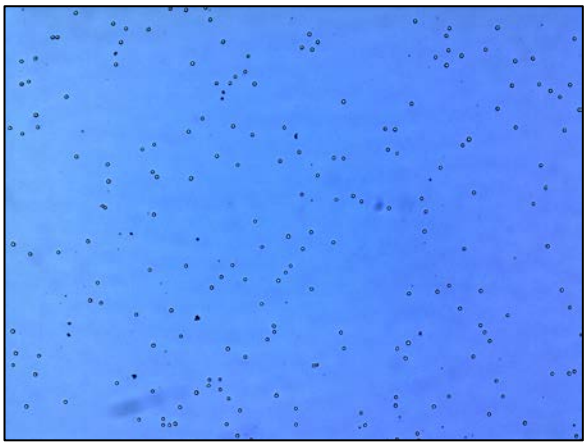
• Cell size histogram expressed by cell concentration

Total cells | Live cells | Dead cells

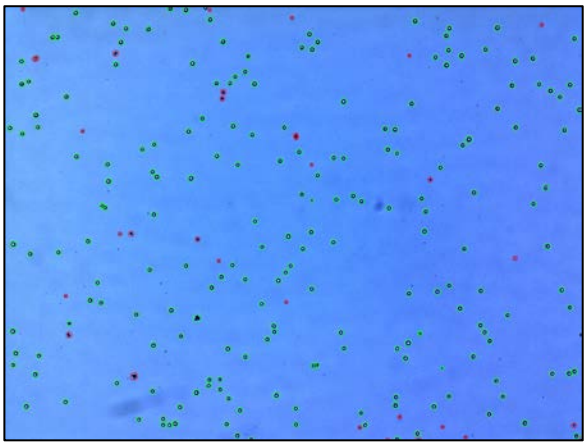
• Cell cluster graph

OLYMPUS

HL-60-08062015_134241_RAW.tif



HL-60-08062015_134241_TAG.tif



A summary of each count performed is automatically saved to the R1.

The R1 stores up to 1000 counts onboard.

Alternatively, press **Print**.

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

Cell Count Report
File name: HL-60-08062015_134241
Date: 08 Jun., 2015 13:42

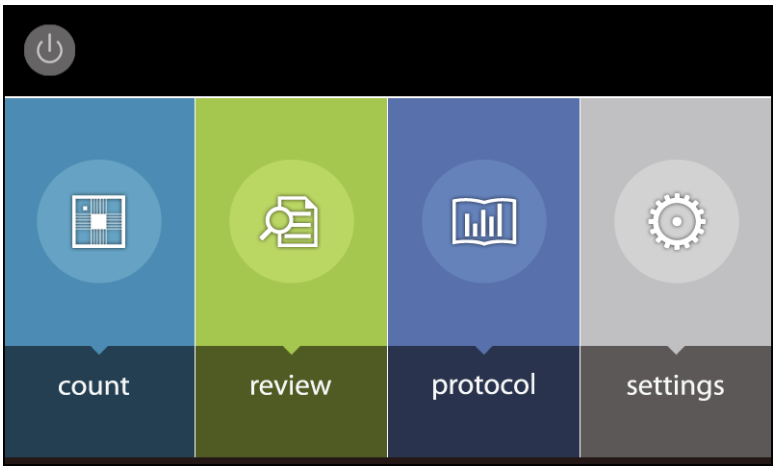
Cell count results
[Total]: 1.06x10e6 cells/mL
[Live]: 9.18x10e5 cells/mL
[Dead]: 1.47x10e5 cells/mL
Viability: 86.2 %
Avg. size: 12.9 µm
Total #: 232 cells
Live #: 200 cells
Dead #: 32 cells
Dil. Factor: 2

Protocol
Protocol name: DEFAULT
Noise reduction: 5
Roundness: 60
Min. cell size: 3
Max. cell size: 60
Size gating: 3 ~ 60 µm

Chapter 5 – Review Previous Results

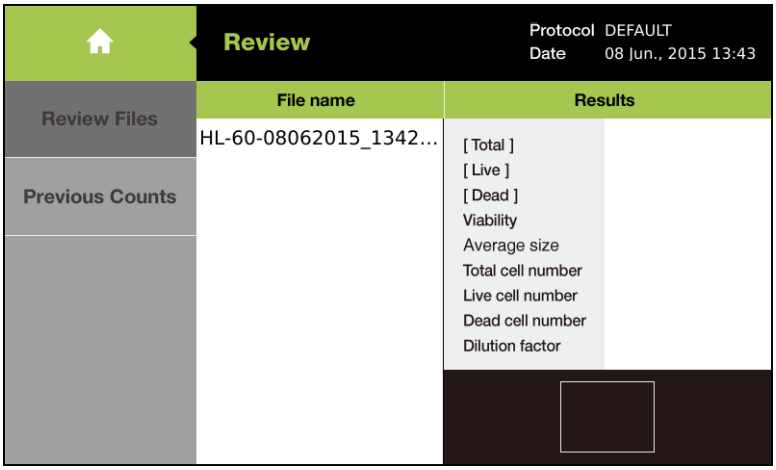
The R1 allows users to review previous results.

Select **review** from the main menu.

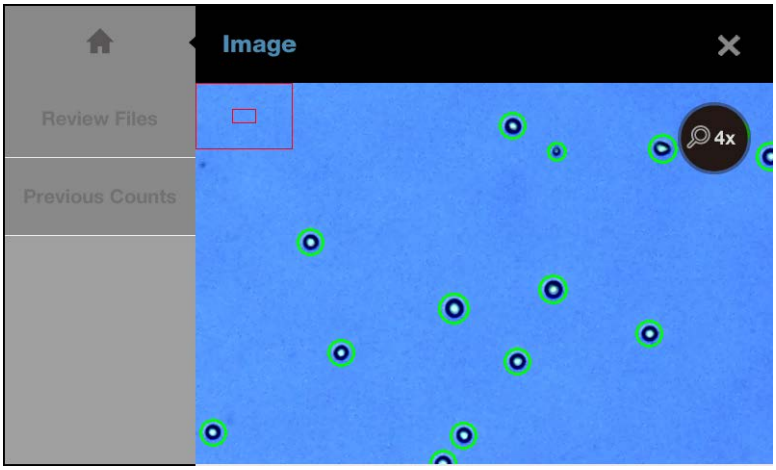


The review screen has two options: **[Review Files]** and **[Previous Counts]**. **[Review Files]** brings up data from a USB memory and **[Previous Counts]** looks up data stored directly on the R1.

Insert a USB memory to the USB port of the R1. Press **[Review Files]** to select an R1-generated folder from the USB memory. The cell count results and corresponding image will appear on the right side of the screen.

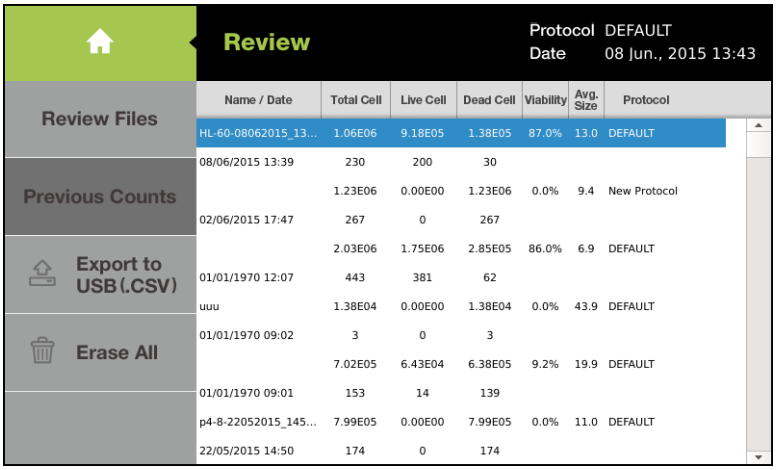


If available in the folder, a tagged image will appear below the results.



The image may be magnified with the magnifier.

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



Chapter 6 – Maintenance and Troubleshooting

6.1 Turning On/Off

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

It is unnecessary to turn the instrument off between uses as standby mode is activated after ten minutes of inactivity. The touchscreen will blackout in standby mode. Simply press the touchscreen or push the power button to start the R1 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 R1 off and disconnect the power cable before cleaning. Ensure 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.

If cell suspension is spilled on the instrument, clean the surfaces with a paper towel or a disposable laboratory wipe dampened with 70% ethanol. If the cell suspension enters the instrument and is unreachable, contact Olympus for assistance.

6.3 Installing Printer Paper

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

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

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

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

6.4 Troubleshooting

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

CHAPTER 7 – POWER CORD SELECTION

If a power cord has not been provided, please select the proper power cord for the instrument by referring to the Specifications and Certified Cords table below.

⚠ CAUTION If you use a non-approved power supply for Olympus products, Olympus can no longer warrant the electrical safety of the instrument.

Specifications

Voltage Rating	125V AC (for 100-120V AC area) or, 250V AC (for 220-240V AC area)
Current Rating	6A minimum
Temperature Rating	60°C minimum
Length	3.05 m maximum
Fittings Configuration	Grounding type attachment plug cap. Opposite terminates in molded-on IEC configuration appliance coupling.

Table 1: Certified Cords

A power cord should be certified by one of the agencies listed in the following table, or comprised of cordage marked with an agency marking per Table 1 or marked per Table 2. The fittings are to be marked with at least one of the agencies listed in Table 1. In case you are unable to buy locally in your country the power supply cord which is approved by one of the agencies mentioned in Table 1, please use replacements approved by any other equivalent and authorized agencies in your country.

Country	Agency	Certification Mark	Country	Agency	Certification Mark
Australia	SAA		Italy	IMQ	
Austria	ÖVE		Japan	JET, JQA	
Belgium	CEBEC		Netherlands	KEMA	
Canada	CSA		Norway	NEMKO	
Denmark	DEMKO		Spain	AEE	
Finland	FEI		Sweden	SEMKO	
France	UTE		Switzerland	SEV	
Germany	VDE		United Kingdom	ASTA,BSI	
Ireland	NSAI		U.S.A	UL	

Table 2: HAR Flexible Cords

APPROVAL ORGANIZATIONS AND CORDAGE HARMONIZATION MARKING METHODS

Approval Organization	Printed or embossed Harmonization Marking (May be located on jacket or insulation of internal wiring)		Alternative Marking Utilizing Black-Red-Yellow Thread (Length of color section in mm)		
			Black	Red	Yellow
Comite Electrotechnique Belge (CEBEC)	CEVEC	<HAR>	10	30	10
Verband Deutscher Elektrotechniker (VDE) e.V.Prüfstelle	<VDE>	<HAR>	30	10	10
Union Technique de l´Electricite´ (UTE)	USE	<HAR>	30	30	10
Instituto Italiano del Marcio di Qualita' (IMQ)	IEMMEQU	<HAR>	10	30	50
British Approvals Service for Electric Cables (BASEC)	BASEC	<HAR>	10	10	30
N.V. KEMA	KEMA-KUER	<HAR>	10	30	30
SEMKO AB Svenska Elektriska Materielkontrollanstalter	SEMKO	<HAR>	10	10	50
Österreichischer Verband für Elektrotechnik (ÖVK)	<ÖVK>	<HAR>	30	10	50
Danmarks Elektriske Materielkontrol (DEMKO)	<DEMKO>	<HAR>	30	10	30
National Standards Authority of Ireland (NSAI)	<NSAI>	<HAR>	30	30	50
Norges Elektriske Materiellkontroll (NEMKO)	NEMKO	<HAR>	10	10	70
Asociacion Electrotecnica Y Electronica Espanola (AEE)	<UNDE>	<HAR>	30	10	70
Hellenic Organization for Standardization (ELOT)	ELOT	<HAR>	30	30	70
Instituto Portugues da Qualidade (IPQ)	| np |	<HAR>	10	10	90
Schweizerischer Elektro Technischer Verein (SEV)	SEV	<HAR>	10	30	90
Elektriska Inspektoratet	SETI	<HAR>	10	30	90

Underwriters Laboratories Inc. (UL)	SV, SVT, SJ or SJT, 3 X 18AWG
Canadian Standards Association (CSA)	SV, SVT, SJ or SJT, 3 X 18AWG

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