

Instruction manual

VWR® Automated Cell Counter Fluo

NA cat. no 49893-2000



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1- Safety Information

Please follow the guidelines below, and read this manual in its entirety to ensure safe operation of the unit.

1.1 General safety requirements

- a) Please follow the instructions on all safety warnings and labels in the manual and on the instrument. The instrument should only be used by professionals within the scope of application, with proper components and accessories suitable for the instrument to be used. The manufacturer will not be held liable for any damage to the instrument caused by any improper operation, including damage to any third party.
- b) Users are allowed to maintain the instrument only to the extent of recommended maintenance stated herein (Refer to Chapter -9 General maintenance) . Any change to the instrument without the manufacturer's authorization may cause failure of the instrument.
- c) In case the instrument breaks down or requires any service, shut down the instrument and contact with VWR customer service.
- d) Use only the fluorescence cubes and power adapter included with the machine, USB port is only for connecting the included USB flash drive.
- e) Working condition: Indoor use only; used in dry location only; Temperature: 10°C~40°C; Humidity: 10%~75% (non-condensing); Altitude: < 2000m; Overvoltage Category II; Pollution Degree 2; Input: DC24V, 1.5A.
- f) Placing requirements: The distance between the back of the instrument and the wall or other equipment is no less than 15cm. The instrument should be installed and placed so as not to cause the user to disconnect the power adapter.
- g) Warranty is only valid when the product is used under normal conditions and in accordance with the instruction manual. The warranty is void if the instrument is not used in accordance with the instructions.
- h) It must be consulted in all cases where  is marked.

1.2 Electrical safety

The following provisions should be followed for the safe use of the electrical devices accompanied with the instrument:

- a) Never disassemble the instrument without any authorization against any possible electric shock.
- b) Check whether the working voltage settings of all components are correct before connecting the instrument to the mains. Use the power supply compatible with the instrument and see the model label of the instrument for the compatible power supply.
- c) Connect the instrument correctly, and ensure it is properly grounded, and the cell counter need to be plugged to a conformly electric and safe system. Any missing or failure to install the fuse, or the failure to connect to any ground wire may pose a risk of electric shock.
- d) Make sure to clean power cords.
- e) If any potential unsafe operation is detected, power off and unplug immediately to disconnect the mains.
- f) If any liquid gets contact with the circuit of the instrument, power off and unplug immediately

- to disconnect the mains, and wipe the moistened parts of the instrument dry in a timely manner.
- g) Keep the bench always dry during operation.
 - h) The fuse type (rated voltage, rated current and model) must comply with the manufacturer's specifications. Do not use any unserviceable or used fuse, and avoid short circuit of the fuse.
 - i) The instrument must be grounded to prevent accidental electric shock.

1.3 Chemical waste safety

Caution! Hazardous Waste. Please refer to the SDS and relevant local regulations for handling and disposing of waste.

To minimize the risk of chemical waste, users must:

- a) Read and understand the SDS provided by the chemical manufacturer, handling or disposal of chemical waste.
- b) Provide main and auxiliary containers for the waste storage. (The main container is used to store direct waste, and the auxiliary container to store waste spilled or leaking from the main container. Both containers must be compatible with the chemical properties of the waste materials and comply with local regulations on waste storage.)
- c) Minimize exposure to chemicals. Wear appropriate protective articles (such as protective glasses, gloves or protective clothing) for chemical handling. For detailed safety guidelines, please refer to SDS.
- d) Minimize the risk of inhalation of chemicals. Close the chemical containers after use immediately. Use chemicals only in a well-ventilated environment (e.g. fume cupboard). For detailed safety guidelines, please refer to SDS.
- e) Handle chemical waste only in a well-ventilated environment.
- f) Use the accompanying cover to seal the waste containers emptied.
- g) Disposal of waste in trays and bottles in accordance with good laboratory practices and local environmental and health regulations.

1.4 Biosafety

It is possible that biological samples (such as samples of tissues, body fluids and blood of human and animals) may transmit infectious diseases. Follow all applicable local regulations from time to time. Wear suitable protective glasses, clothing and gloves.

1.5 Certificates

Test Standards:

UL/CSA 61010-1, 3rd Edition
UL 61010-2-081, Third Edition/CSA 61010-2-081:19

2- Package Contents

Configuration	Part numbers	Items	Quantity	Purpose Description
Standard	49893-2000	VWR® Automated Cell Counter Fluo	1	Cell count
Standard	/	AC adapter (NA)	1	Power supply
Standard	10228-0050	VWR® Cell Counting slides	1	1 box of 50 pcs
Standard	/	USB flash driver	1	Data transfer
Standard	/	Slotted screw driver	1	Replace the fluorescence cube
Optional	49893-4951	VWR® Fluo Cube Green GFP	1	3 fluorescence cubes are available separately for selection to provide excitation light sources of different wave lengths
Optional	49893-4952	VWR® Fluo Cube Red RFP-PI	1	
Optional	49893-4950	VWR® Fluo Cube Blue DAPI	1	

3- Intended use

This equipment is designed for measurement and further fluorescence analysis of cell quantity and viability for cell lines, stem cells and primary cell samples. For research only. Not intended for diagnostics use.

4- Symbols and conventions



Flammable environment hazard

Never operate the instrument in an environment with flammable gases.

Danger pour l'environnement inflammable

Ne jamais utiliser l'instrument dans un environnement contenant des gaz inflammables.



Electromagnetic interference hazard

Make sure to operate the instrument in a controlled electromagnetic environment to avoid any risk associated with instrument failure.

Risque d'interférence électromagnétique

Assurez-vous de faire fonctionner l'instrument dans un environnement électromagnétique contrôlé pour éviter tout risque associé à une défaillance de l'instrument.



Electrical safety

Do not disassemble the instrument. Power off before maintenance. OPERATORS must be trained before opening rear cover and replacing fluorescence cubes.

Sécurité électrique

Ne démontez pas l'instrument. Mise hors tension avant entretien. Les opérateurs doivent être formés avant d'ouvrir le couvercle arrière et de remplacer les cubes de fluorescence.



Radiation hazard

Follow all applicable radiation safety procedures all the time when handling radioactive samples. Make sure to take appropriate disinfection and safety measures during disposal of radioactive pollutants. Operators must always wear protective supplies (such as particle protective mask, gloves, protective shoe covers) according to the relevant rules and regulations of the respective laboratories for the disposal of radioactive pollutants which must be performed in accordance with the relevant regulations.

Risque d'irradiation

Suivre toutes les procédures de radioprotection applicables en tout temps lors de la manipulation d'échantillons radioactifs. Assurez-vous de prendre les mesures appropriées de désinfection et de sécurité lors de l'élimination des polluants radioactifs. Les opérateurs doivent toujours porter des fournitures de protection (telles que masque de protection contre les particules, gants, housses de chaussures de protection) conformément aux règles et règlements applicables des laboratoires respectifs pour l'élimination des polluants radioactifs qui doivent être effectuées conformément aux réglementations en vigueur.



Biological infection hazard

Samples used in the intended operation of the instrument may be infectious. For this reason, it is recommended to follow the general laboratory regulations on infection control procedures. For information on decontamination agent, its use, dilution and effective application, please refer to the Laboratory Biosafety Manual issued by the World Health Organization in 2020. Follow all applicable safety procedures all the time when handling infectious samples. Make sure to take appropriate disinfection and safety measures during disposal of infectious substances. Operators must always wear protective supplies (such as particle protective mask, gloves, protective shoe covers) according to the infection control procedures of the respective laboratories, and infectious pollutants must be disposed of in accordance with the relevant regulations.

Risque d'infection biologique

Les échantillons utilisés dans le fonctionnement prévu de l'instrument peuvent être infectieux. Pour cette raison, il est recommandé de suivre le règlement général des laboratoires sur les procédures de contrôle des infections. Pour obtenir des renseignements sur l'agent de décontamination, son utilisation, sa dilution et son application efficace, veuillez consulter le manuel de biosécurité en laboratoire publié par l'organisation mondiale de la santé en 2020. Suivez toutes les procédures de sécurité applicables en tout temps lors de la manipulation d'échantillons infectieux. Assurez-vous de prendre les mesures appropriées de désinfection et de sécurité lors de l'élimination des substances infectieuses. Les opérateurs doivent toujours porter des fournitures de protection (comme un masque de protection contre les particules, des gants, des couvre-chaussures de protection) conformément aux procédures de contrôle des infections des laboratoires respectifs, et les polluants infectieux doivent être éliminés conformément à la réglementation pertinente.

5- Product Specifications

Parameter	Description
Cell size range	Recommended 7-60 μm , allowable 4-60 μm
Cell circularity range	1-100 (min./max. size)
Cell concentration range	10^4 - 10^7 cells/mL
Cell counting time	<9 s
Accuracy of multi-recognition	CV<5%
Built-in memory	1,000 entries
Touch screen resolution	1024*600
Operation response time	100 ms
Optics	5 megapixel camera, 2.5X optical magnification
Fluorescence cube (optional)	DAPI(375/28Ex 460/50Em) AO/GFP(480/30Ex 535/40Em) PI(540/25Ex 620/60Em)

6- Instructions for use

6.1 Overview

In the process of cell culture, it is necessary to determine the cell concentration and identify the cell viability to guide the subsequent experiments. For some more complicated cell experiments or samples, fluorescence analysis is needed to distinguish and count the quantity of a certain type of cell. For example, count the quantity of cells expressing GFP fluorescence in the transfection experiment.

The automated cell counter collects and analyzes cell images and then obtain the concentration of the sample. After trypan blue staining, the proportion of living/dead cells can be obtained. Specific fluorescence analysis can be performed after the fluorescence cube is installed.

6.2 Description of Buttons and Switches



Fig. 6-1

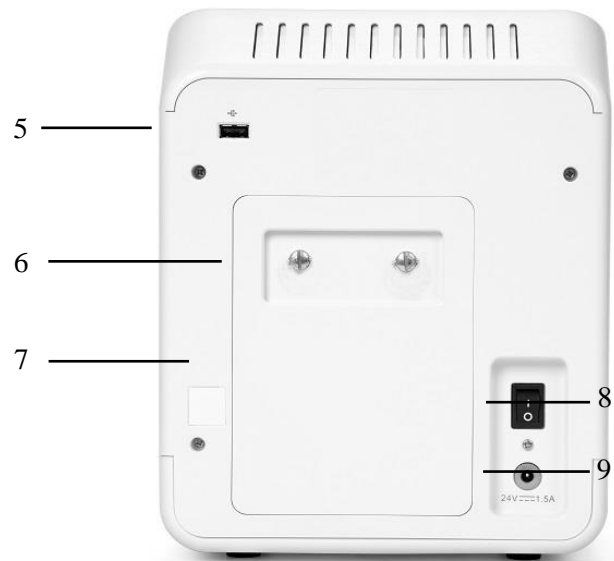


Fig. 6-2

- ① Touch screen display: 7-inch capacitive screen displaying user operation interface.
- ② POWER Indicator.
- ③ USB port 1: allows users to download the cell count data and image onto an external device with the USB port for storage. USB flash drive format: FAT32.
- ④ Counting slide port: allows users to insert the counting slide loaded with samples. Press the inserted counting slide until it pops out, then replace it with a new one.
- ⑤ USB port 2 : the function is the same as USB port 1.

Note: two USB ports are provided at the front and rear of the instrument for data transfer, and the instrument does not support reading of two USB flash drives simultaneously.

- ⑥ Lock screw on rear cover plate: users are allowed to loosen two lock screws counterclockwise with the supplied slotted screw driver.
- ⑦ Rear cover plate: allows users to use fluorescence cubes (optional) and store fluorescence cube tools.



Note: The instrument must be powered off before opening the rear cover plate.

- ⑧ Power switch: main power switch.
- ⑨ Power inlet: connect one end of the provided power adapter to the power supply and the other end to the power inlet.

7- Operation

7.1 Start-up

When the instrument is properly wired, users are allowed to start up the cell counter. Press the power switch to start up the instrument and enter the main interface as shown in the picture below.

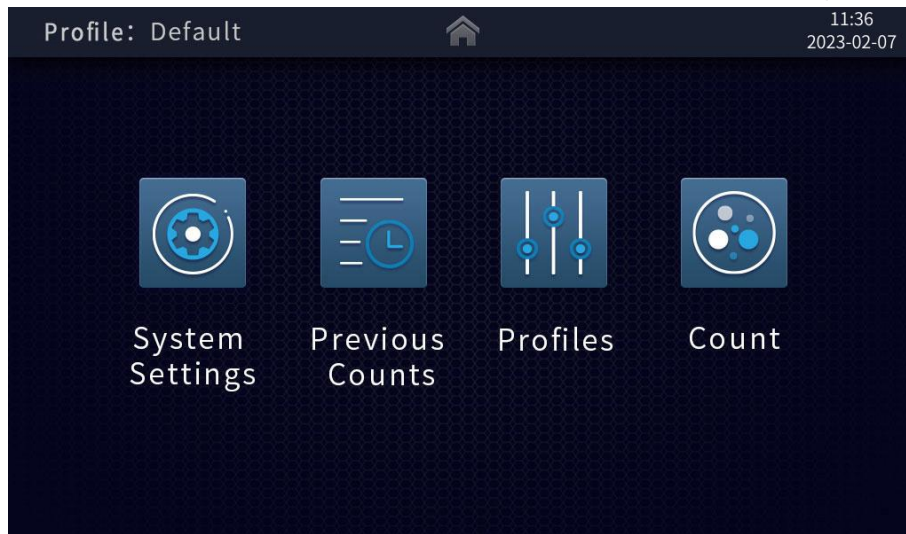


Fig. 7-1

The main interface contains four parts: System Settings, Previous Counts, Profiles, and Count.

7.2 Profiles

In the bright field (BF) assays, users are allowed to set the threshold values of the count results based on the profile sliders of size, brightness and circularity; in the fluorescence field (FL) assays, users are allowed to adjust the RFU slider as required to set the threshold values of the count results.

The Profiles interface allows users to adjust the threshold values of the profiles to be used, rename and save them for calling of such adjusted Profiles in the next cell counting.

Bright field (BF) Mode

Click [Profiles] on the main interface to enter the profiles setting interface, with the instrument in Non-Trypan Blue Mode (Non-TP) by default under which the total cell concentration, histogram of total cell size distribution histogram and average size are available for view.

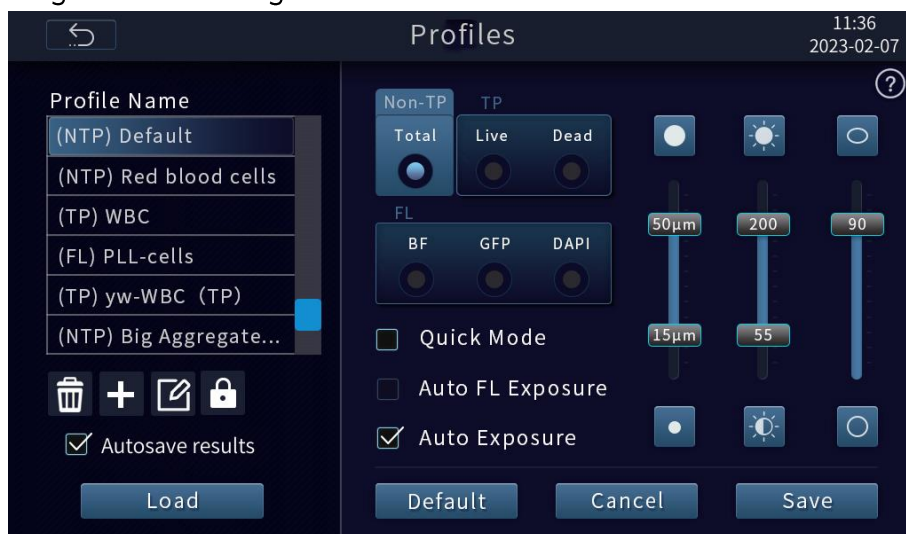


Fig. 7-2

Click [Trypan Blue] (TP) to switch to Trypan Blue mode under which the total cell and dead/ live cell concentrations, histograms of dead / live cell size distribution and average size are available for view.

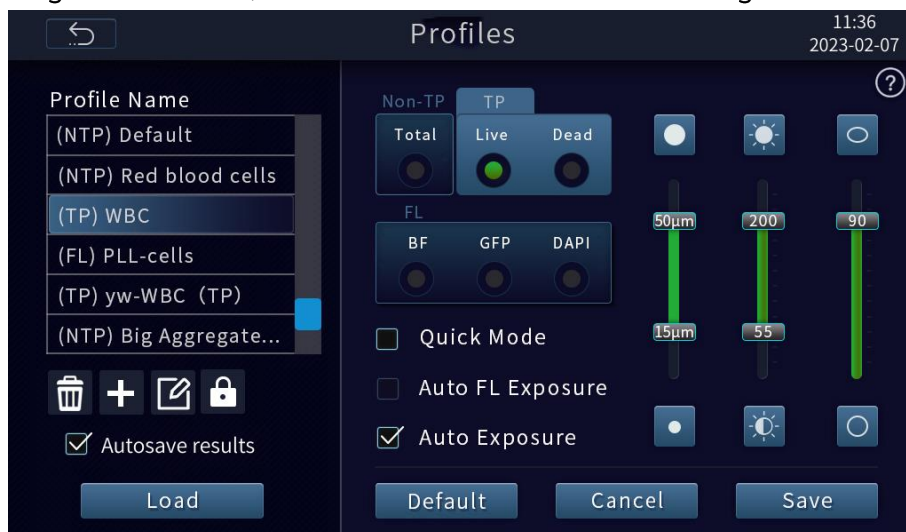


Fig. 7-3

Fluorescence field (FL) Mode

The following figure shows the adjustment of the fluorescence threshold values. The count result in this mode includes the total cell recognition chart, total cell concentration, AO/GFP, DAPI, PI cell concentration, total cell size/quantity distribution histogram, fluorescence intensity/cell quantity distribution histogram, etc.

Note: if the BF channel is only selected on the Count interface in the FL mode, only the total cells are available for calculation, and fluorescent cell will be undistinguishable.

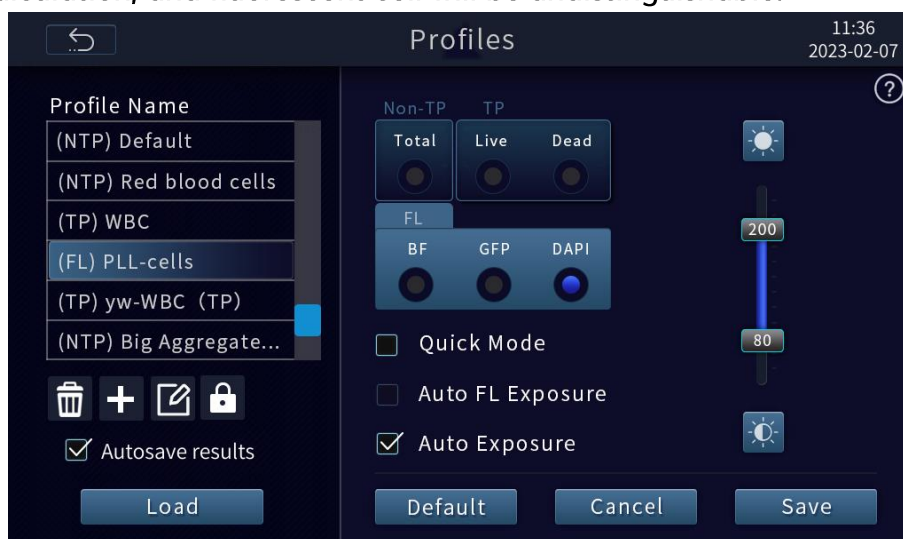


Fig. 7-4

- **Parameter adjustment**



Size, brightness and circularity sliders

Size: drag the slider to enable the system to screen the cells by the size while counting.



Size screening: allowable range of 7-60µm by default, the lower limit of identification up to 4µm;

When there are much debris in the sample, the interference of cell debris can be eliminated by adjusting the recognized size range and setting the threshold value.

Brightness: drag the slider to enable the system to screen the cells by brightness while counting.

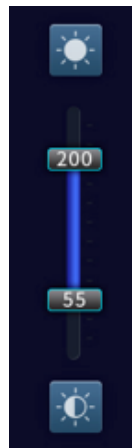


Circularity: drag the slider to enable the system to screen the cells by circularity while counting.



The circularity slider can only be used to set a single threshold value; cells within the set value range will be counted, excluding the cells beyond the range. Drag the slider toward the target to adjust the circularity threshold.

RFU : drag the slider to enable the system to screen the cells by RFU while counting.



RFU slider



= Target with higher RFU



= Target with lower RFU

RFU ranges from 0 to 255, and in general it is not necessary to make adjustments in the profiles. After imaging, the device will automatically calculate the RFU of the background, and set the lower limit of RFU above the background, so as to filter it.

The automatically calculated background can usually be distinguished from the fluorescent cells, but its threshold value will fail in the event of too strong the fluorescence background or too weak cell fluorescence, possibly leading to the removal of the weak cell fluorescence or the presence of residual background fluorescence. Then, the RFU threshold needs to be adjusted to achieve the best effect of background removal and fluorescent cells retaining.

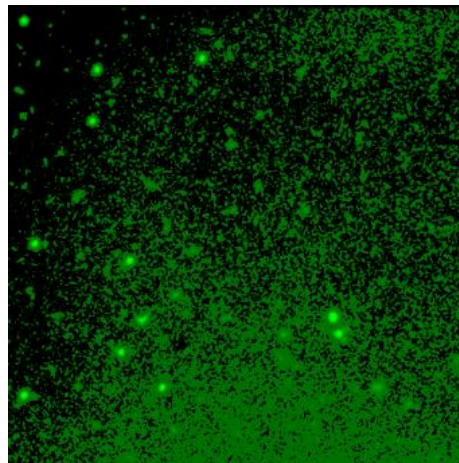



Fig. 7-5

Too strong fluorescence background leads to residual background

Click or hold down the above icon to adjust each parameter threshold.

Click the [Help] symbol  in the upper right corner of the interface to learn more about the concepts of size, brightness, circularity, RFU and so on as shown in figure 7-6.

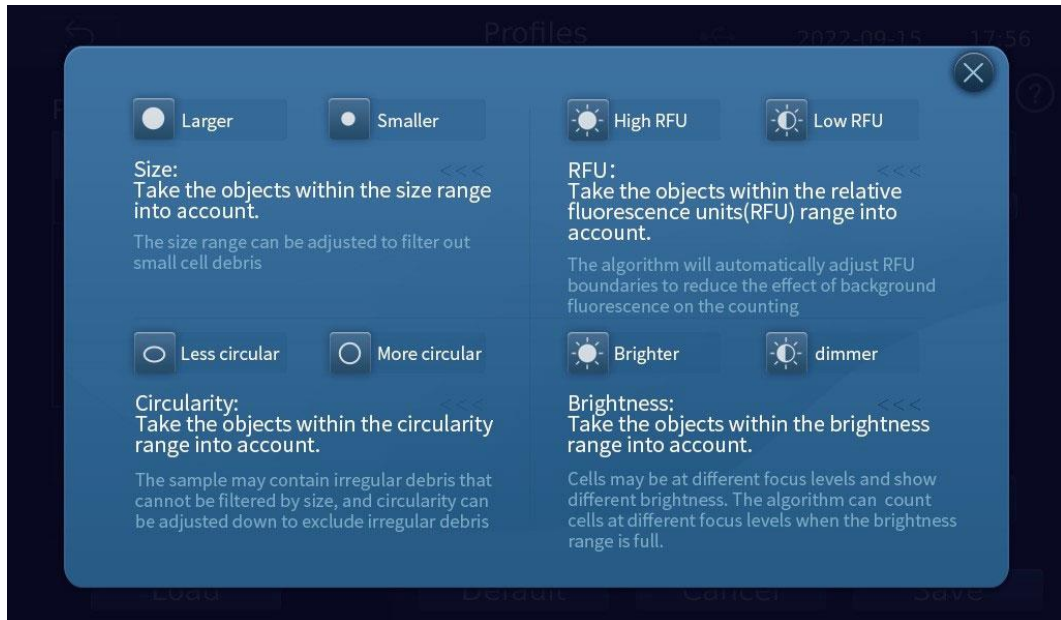


Fig. 7-6

Quick Mode

By checking [Quick Mode], you will perform count in a quick mode; otherwise, you will perform count in a standard mode. After selecting the count parameters in the Quick mode, once the counting slide is recognized, the number pad pops up for user to enter a Profile name, and then the system will count the cells and output the results; in the Standard mode, the focusing results will be presented first, and then the count results will be output after manually clicking the [Count] key.

The quick mode is suitable for cell samples with no debris and moderate concentration. It can automatically focus and count after inserting the counting slide, which can greatly reduce the counting time. Even if the imaging effect in the counting results is not ideal, you can click the back key to enter the Count interface again for manual fine-tuning.

Note: due to complex fluorescence applications, Quick Mode is not recommended for fluorescence counting.

Auto Exposure and Auto FL Exposure

Users are free to choose to activate [Auto Exposure] or [Auto FL Exposure], of which the Auto Exposure features allow users to adjust the BF/FL brightness respectively depending on the characteristics of cells for count, and then choose whether to restore the system to the default brightness after counting.

Autosave results

When [Autosave results] is checked, the counting is complete and the counter exits, the data can be saved automatically.

Note: Autosaving succeeds only when the local storage is sufficient.

Restore to Default Mode

Click [Default] to restore part of the initial settings, including Auto FL Exposure, Auto Exposure, Quick Mode, Standard Mode, threshold value of each profiles, etc.

7.3 Profiles Loading

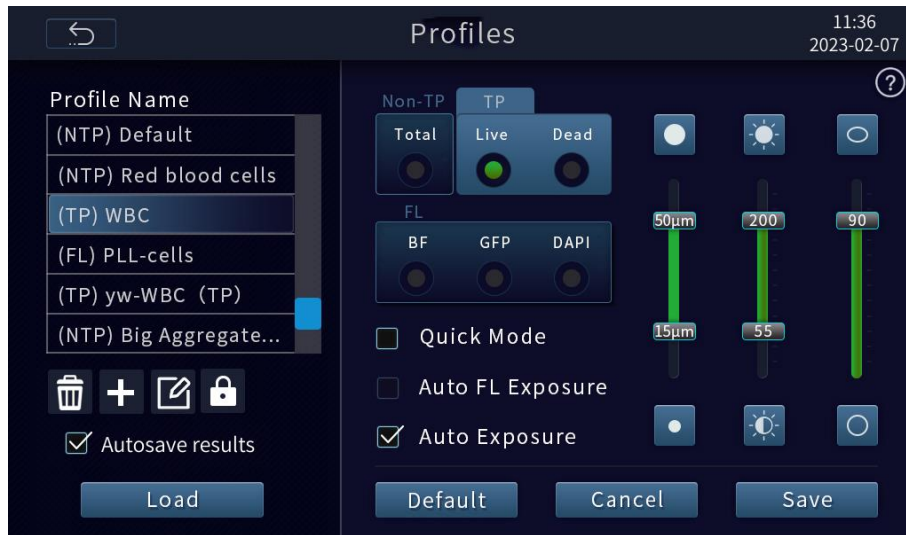








Fig. 7-7

In the left column of the Profiles interface, you can delete/ add/ rename/ Lock profiles by clicking the icons of     respectively.

Note: Locked parameters cannot be edited.

Click lock key  to lock the selected profile, the icon is also displayed on the right of the profile name; if you want to unlock the profile name, select it again, the lock key at the bottom becomes the unlock key , click the unlock key and confirm as prompted. Here, in addition to the "Default" profiles, up to 17 profiles can be added, which can be called directly before and during count.

Each profile defines the count parameters (size, brightness, circularity and fluorescence intensity) to simplify the workflow. After selecting a profile, click [Load] to apply the specified count parameter to all new cell counts. Users already completing counting, are allowed to apply the new count settings to the current count results (total cells, viability, etc.) and all new counts by loading new data from the Results interface.

7.4 Sample Preparation

The instructions below should be followed for the best results:

- 1) Make sure to mix the cell suspension well before drawing the cell sample. After the cell sample has been standing for a long time, it is necessary to flick the tube or pipette the cell suspension gently and draw the sample from the centre of the tube rather than its bottom.
- 2) Cell concentration range: 1×10^4 – 1×10^7 cells/mL, optimally 1×10^5 – 4×10^6 cells/mL.
- 3) Make sure that the count area is covered with cell suspension to get accurate cell viability analysis results and start counting immediately after staining according to the analytical protocol.
- 4) Do not press the optical surface of the counting slide, but hold it by the edges.

- 5) Carefully avoid any bubble formed in the sample.
- 6) Minimize the amount of debris in the cell sample to the greatest extent. No matter which counting or staining method is selected, it is difficult to count and assess the viability of samples containing large amounts of debris which may come from trypan blue stain or cell samples. Possible treatments for different sources of debris are provided as follows:
 - ◆ Centrifuge trypan blue solution and take the supernatant;
 - ◆ Centrifuge and re-suspend cell suspension.

7.5 Methods of Staining

Tips: If you only need to count the total cell concentration, you could directly load sample without staining.

• Trypan blue staining

Recommended staining method:

- 1) Mix 10 μ L cell suspension with 10 μ L 0.4% Trypan blue solution.
- 2) After pipette and mixing well, transfer 10 μ L of stained cells into the cell counting slide for analysis.

• AO/PI Staining

Recommended staining method:

- 1) Staining according to the recommended method and concentration in the PURCHASED AO/PI kit. In most cases, when the final concentration of the stain solution is 2-20 μ g/mL, the cell samples incubated for 5-30 minutes will produce bright nuclear staining fluorescence. However, in certain cell types, conditions, and applications, optimization is required. At this point, the concentration of stain solution can be increased or decreased for the best staining strength.
- 2) Rapid staining:
 - ◆ Prepare a staining premix of 10 μ g/mL AO and 50 μ g/mL PI in advance.
 - ◆ Transfer 20 μ L cell suspension into tube, and add AO/PI premixed staining solution of the same volume (the recommended AOPI final concentration is AO: 5 μ g/mL, PI: 25 μ g/mL).
 - ◆ After pipette and mixing, incubate for 3-5min.
 - ◆ After pipette and mixing well, transfer 10 μ L of stained cells into the cell counting slide for analysis.

• DAPI Staining

Recommended staining method:

Staining according to the recommended method and concentration in the PURCHASED DAPI kit. In most cases, when the final concentration of the DAPI stain solution was 0.1-1 μ g/mL, the cell samples incubated for 3-10 minutes will produce bright nuclear staining fluorescence. However, in certain cell types, conditions, and applications, optimization is required. At this point, the concentration of stain solution can be increased or decreased for the best staining strength.

Note: since DAPI stain solution is semi-permeable and can stain both live cells and fixed cells, longer staining time for live cells is required, otherwise the proportion of stained cells will be lower. For avoidance of longer waiting, users are allowed to add 75% alcohol of the same volume to kill the cells for staining if don't need to count the cell viability.

7.6 Loading Samples into Slide

- 1) After pipette and mixing well, transfer 10 μ L of sample into the cell counting slide for analysis.
- 2) Allow the sample to stand for 30 seconds in the slide, and insert the sample-loaded slide into the slide port. When correctly inserted, a soft click will sound.
- 3) After counting, just push the counting slide gently into the slide port until it clicks and is pushed out under spring pressure. Hold the counting slide by the edges to take it out.

Note: do not reuse the disposable counting slide, which may influence the count results.

7.7 Bright Field Assays

If the system recognizes insertion of the counting slide, the file name editing interface will pop up. Shown as figure 7-8.

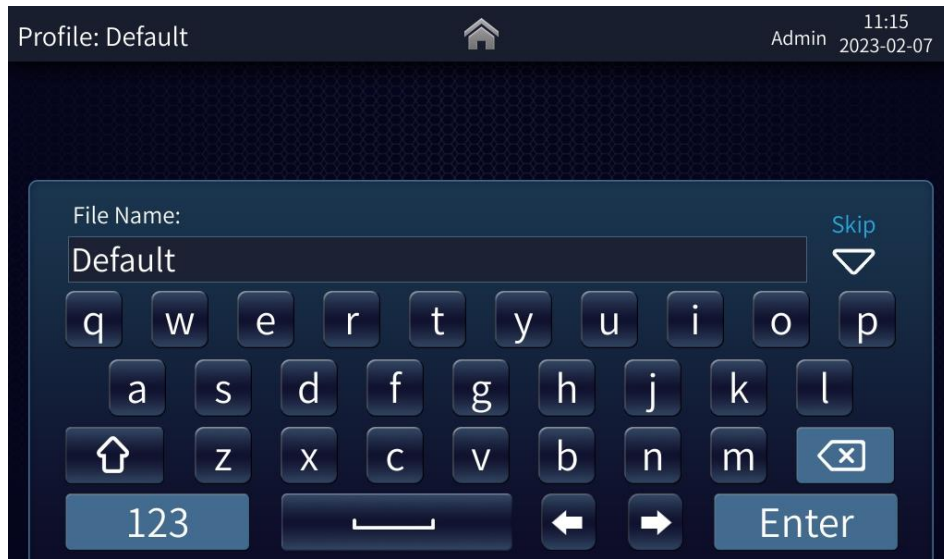


Fig. 7-8

Enter the file name and click [Enter] or click [skip] ("skip" means that the default name or last typed name is selected) to enter the count interface. Here you could start counting and perform such actions as zooming in, fine tuning of focus and light source brightness adjustment, so as to capture optimized images for accurate count of cell concentration or dead/ live cells.

Count page of TP/Non-TP mode:

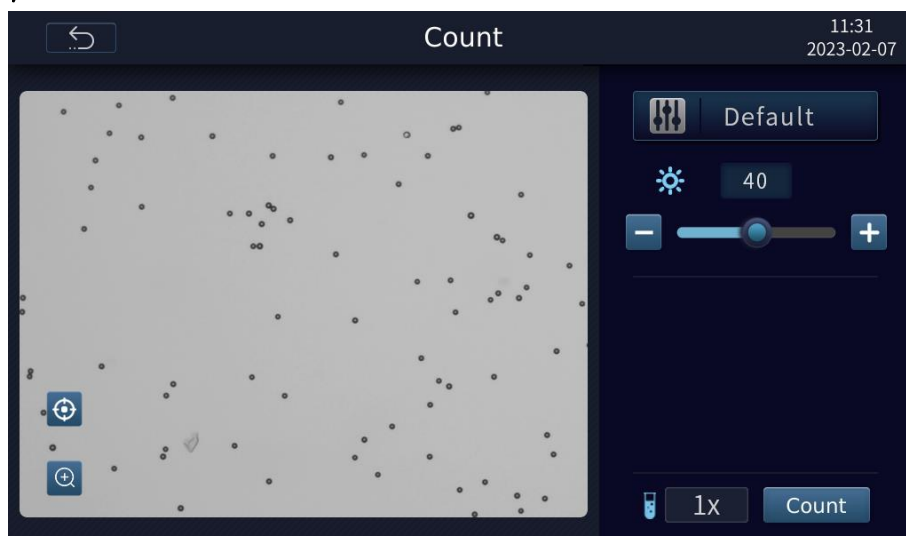


Fig. 7-9



Default

in the figure above shows the active profile file name. Click this icon to enter the Profiles page.






1x

For setting the dilution factor of the sample. Click "1x" to pop up a numeric keypad. The range of setting value: 1.00~999.00.

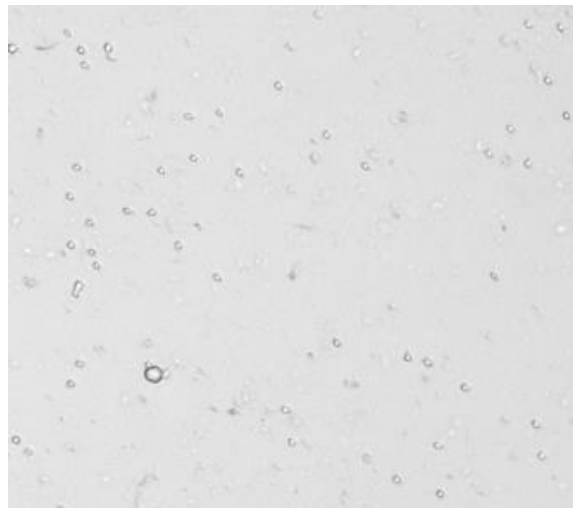
Note: The default dilution of Trypan Blue mode is "2x", and the default dilution of "AO+PI" mode is "2x".

Dilution factor of sample:

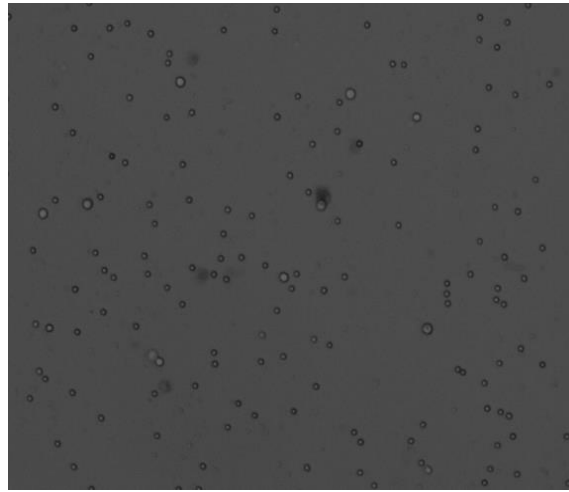
The cell concentration counted by cell counter is the measured concentration of the cells on the current counting slide, which may be accessible from the dilution of the original solution; so users need to convert the current concentration into the concentration of the original solution to calculate the volume of the original solution required for dilution to the target concentration.

 allows users to adjust the light source brightness. If users check [Auto Exposure], the results will be auto adjustment values by default. Users can also manually adjust the brightness of light source by clicking  & . Adjustment range: 0-100.

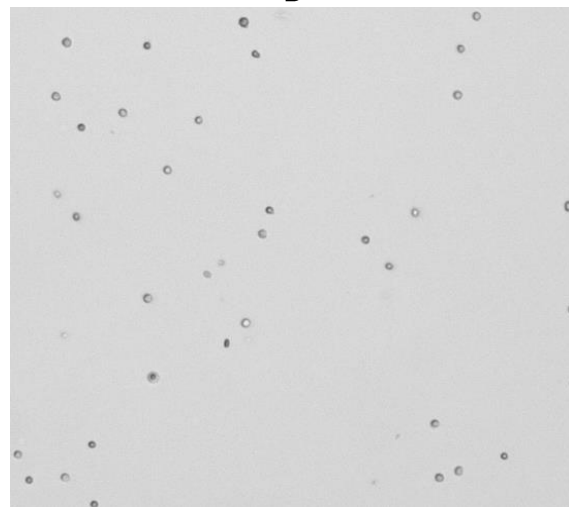
Brighter or dimmer effect may have an effect on the imaging quality. In general, when automatic exposure is activated, better bright field white light illumination can be obtained automatically on the imaging interface. The overexposed, moderate and underexposed effects of the bright field are as shown in Fig. 7-10 (A-C) below.



A



B



C

Fig. 7-10



- A. Overexposure: brighter light source, whiter background, and the cell details shaded by light, affecting cell recognition.
- B. Underexposure: dimmer light source, darker background, and darker cells as a whole, affecting the judgment of the live and the dead cells.
- C. Normal exposure: moderate brightness of light source, clear outline of cells, live and dead cells showing significantly different transmittance characteristics.

Focusing:

When the counting slide is inserted, the system recognizes the counter and starts focusing automatically.

Click  to pop up the slide bar: 

Click [Auto] to execute auto focus.

Click  and  to adjust the focus. Focus value is displayed in real time, such as "1230" showed above the bar. Adjustment range of aggregation value: 0-2400.

The mean of [Set]: customize the focus value. The next time the instrument is used, the system will focus based on the focus value of the last "Set" in order to quickly obtain good pictures for accurate counting.

Note: the point of set will be influential to autofocus.

Focusing recommendations:

It is critical to ensure proper focus to accurately identify cells and distinguish dead or live cells. Cell images with correct and incorrect focus are as shown in Figure 7-11(A-B)

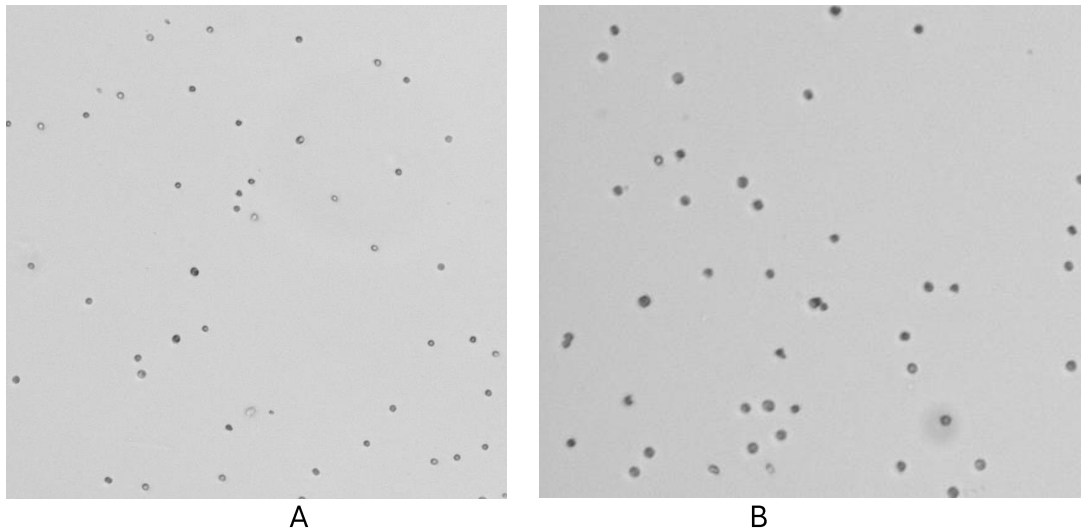


Fig. 7-11

A Normal focus: well-outlined cells, with a clear aperture in the middle of live cells, and opaque dead cells due to trypan blue staining.

B. Abnormal focus: blurred cell contour, presented as opaque dead cells.

Two possible results from incorrect focusing:

- Due to the spherical structure of the cell, the cell center will present as opaque after as if it had been stained with Trypan blue in the event of deviation of the focal length of the device, leading to the misjudgement of live cells as dead cells by the device.
- The focal length of the device is deviated, and the cells presented a fuzzy halo, which causes the device to misjudge the dead cells as live cells.

Determination of correct focusing:

- After automatic focusing, observe the imaging state of the cells in the field of vision. If some cells show clear outline and is transparent in the middle part, and some cells are clear as a whole but opaque in the middle part, then the cell focus is correct.
- If there are no obvious features mentioned above, appropriately enlarge the image and select the area with a large quantity of cells, and slightly adjust the focal length to observe the changes of cell imaging in a clear manner when the focal length changes and select the state with the clearest cell edge as the focus.

By clicking  , the slide regulator  will show up to allow users to zoom in/out the active view/ image.

Upon completion of operations on the [Count] page, the [Result] page will pop up, where users are allowed to zoom in, mark cells, or view histograms, adjust profiles and count for the captured images. The results page of TP Mode as shown below.



Fig. 7-12

Click  to pop up a histogram, as shown in the figure below, and click it again to exit.



Fig. 7-13




Click  to mark cells, as shown in the Fig.7-14 below.



Fig. 7-14

Click  to enter the Adjustment page to re-adjust the profiles.

Click  to enter the Dilution Calculator page, as shown in the figure below, where users are allowed to calculate the volume of existing cell stock solution and the volume of buffer solution to be mixed and diluted to the target volume and concentration.

 is a "Help" symbol on the upper left corner of the interface; click it for more about calculation method.

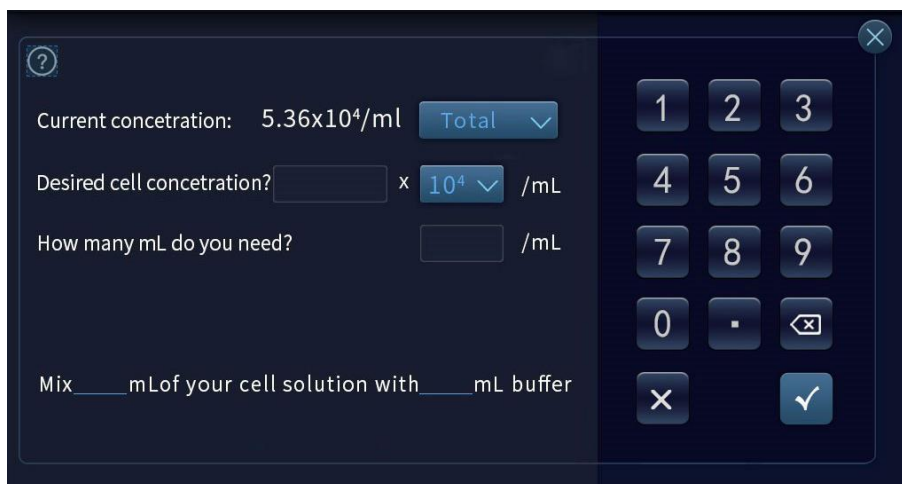


Fig. 7-15

7.8 Fluorescence Assays

The following figure shows the data acquisition page in FL assays. This page allows users to perform such actions as amplification, focused fine-tuning and light source brightness adjustment, so as to capture optimized images.

Note: for the icons and their functions in FL assays, please refer to the corresponding introductions of BF assays.

Click to switch to the data acquisition page of FL assays

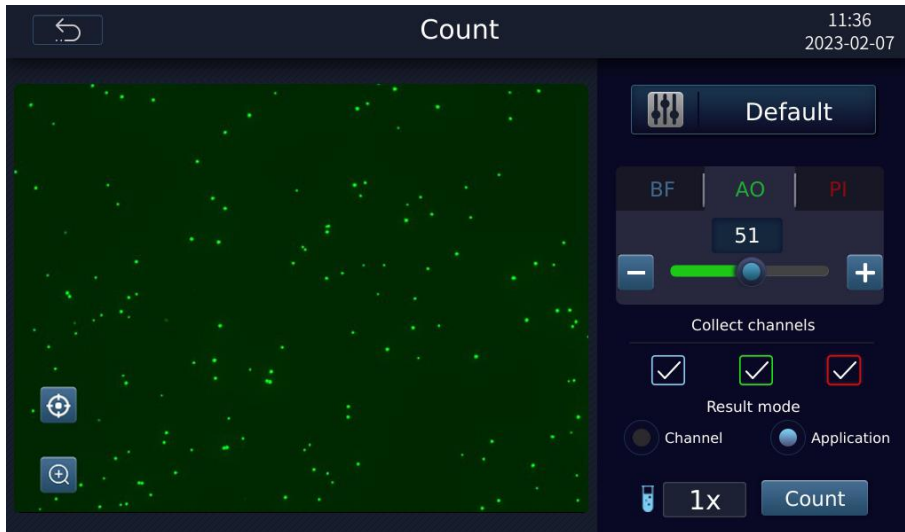



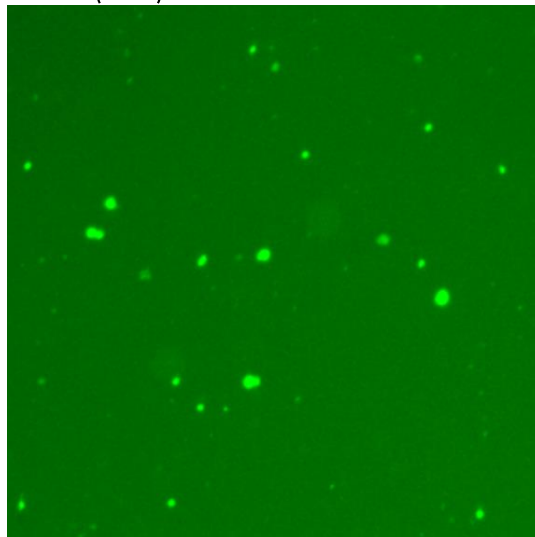


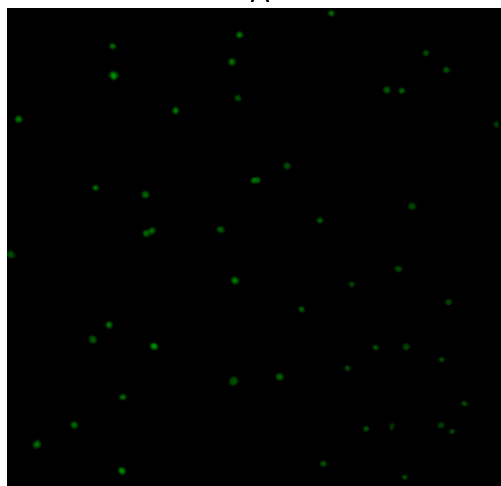
Fig. 7-16

 allows for the adjustment of brightness. If the [Auto FL Exposure] is checked, the default value will be automatically adjusted value, and the brightness can also be manually adjusted by clicking  and . Adjustment range: 0-100.

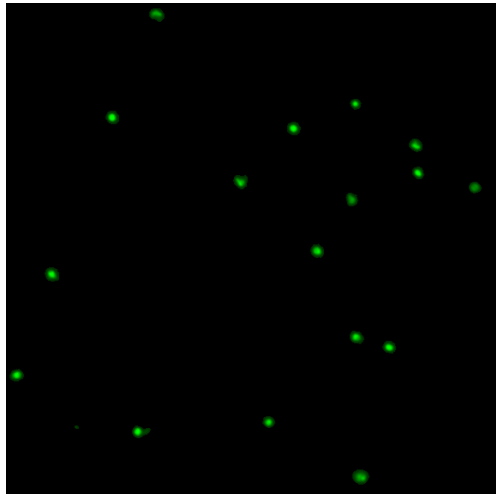
Since the application of fluorescence is complex, the result of auto FL exposure is affected by the RFU and the quantity of fluorescent cells. The result of the overexposure, moderate and underexposure of fluorescence is as shown in Figure 7-17(A-C)



A



B



C

Fig. 7-17

- A. Fluorescence overexposure: brighter fluorescent light source and too strong fluorescence background=, affecting cell recognition and observation.
- B. Underexposure of fluorescence: dimmer fluorescent light source, black overall fluorescence of the cells, darker overall fluorescence of the cells, and some cells with lighter staining missing in counting.
- C. Moderate fluorescence exposure: moderate brightness of fluorescent light source, no obvious background, uniform fluorescence brightness of cells, clearly visible.

Description on Channel/Application:

When the light source is a combination of AO&PI or GFP&DAPI, and [Quick Mode] is selected, [Application] is automatically used to output the results, and other combinations of light sources will output the results through [Channel].

Take AO & PI for an example:

Results page of [Channel]



Fig. 7-18

Results page of [Application]



Fig. 7-19

 is a channel button; the channel menu will pop up when it is clicked.

Click  to mark cells.

The channel menu is only displayed in the FL assays. Users can switch the light source channel to check the fluorescence status of fluorescent stained cells under different light source states.


Click  to get a histogram, as shown in the figure below. Users are free to view the histogram in the BF assays or FL assays, of which the histogram in the BF assays is in cell size-based distribution, while the histogram in the FL assays is in RFU intensity-based distribution.



Fig. 7-20

Refer to the BF assays for relevant functional buttons.

7.9 Results Storage

After counting, users are allowed to save or export the original image, cell marking image, count profiles, cell count report and other data, and are also allowed to preset the default naming rules for results exporting.

Click [Save as] to save the results to the local Storage in various forms or/ and to an acceptable external storage device. Click [Done] to enter the [System Homepage] and automatically save the results to the local computer or/ and external storage device under the system default name.

Note: 1000 entries are available for storage in the system at most. If users want to save new data beyond this limit, the earliest count results saved will be deleted by default.

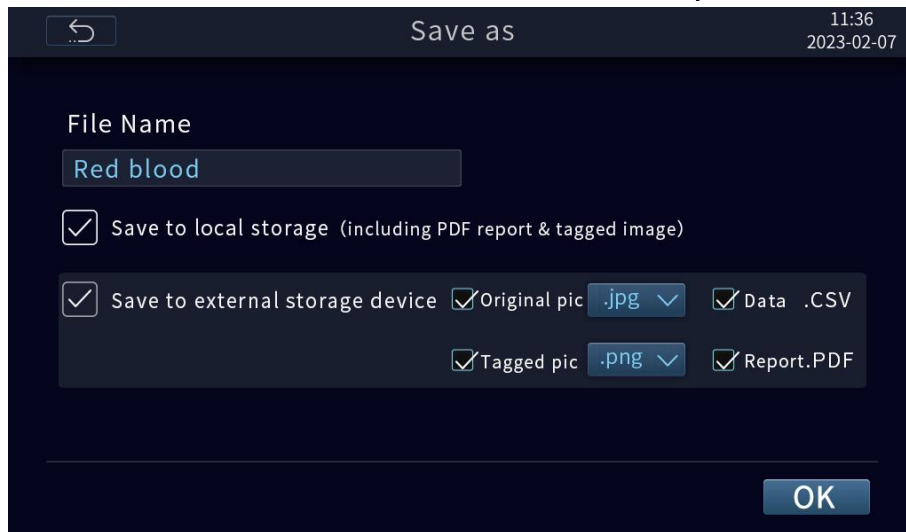


Fig. 7-21

7.10 Previous Counts

Click [Previous Counts] on the Homepage to enter the following interface, where users are allowed to view the previous data of the instrument operation, delete and export data to the external storage device.

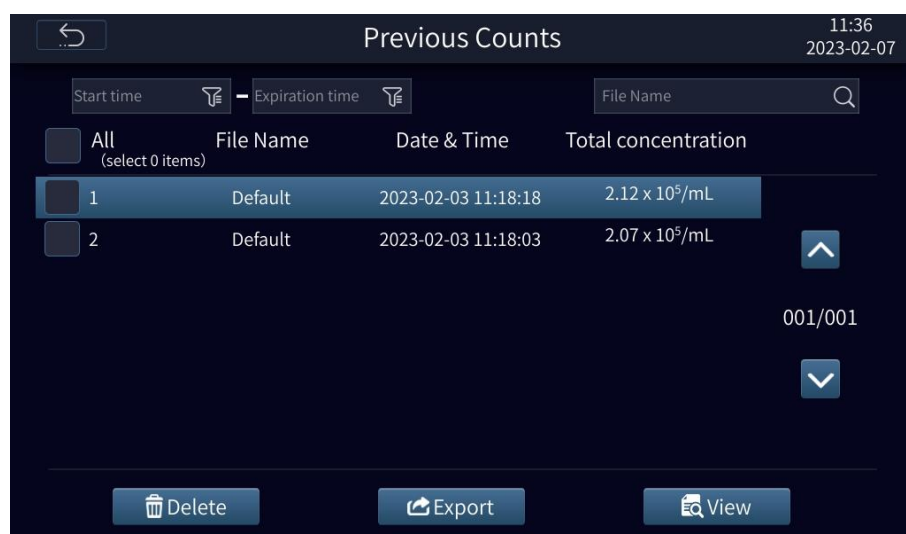


Fig. 7-22

7.11 System Settings

Click [System Settings] on the Homepage to enter the following interface. It allows users to perform such actions as software update, date / time setting, language switching, fluorescence cube resetting (for replacement of fluorescence cube).

Note: "Maintenance" is only for after-sales personnel.

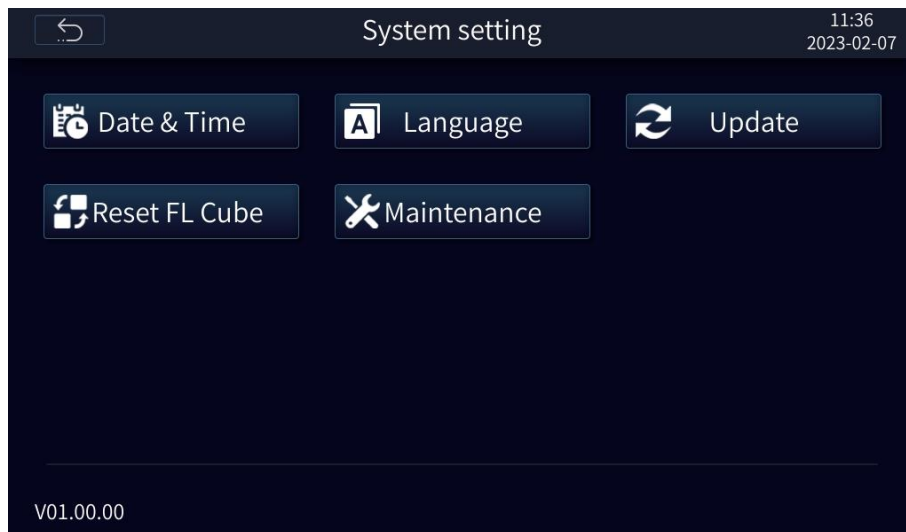


Fig. 7-23

- **Software update**

The latest version of the software is available for updating to the instrument by the USB flash drive.

Method: First copy the latest software to the USB flash drive, insert the USB flash drive into the USB port, when the USB flash drive is successfully recognized, you could click [Update Now] .

When the software program in the USB flash drive is successfully recognized, the keyboard pops up.

After the user enters the correct password, the software update interface is displayed. Please follow the prompts.

Note: the .CSV data in the USB flash drive will be automatically deleted after upgrading. It's recommended to back up the data to the PC before the upgrade.

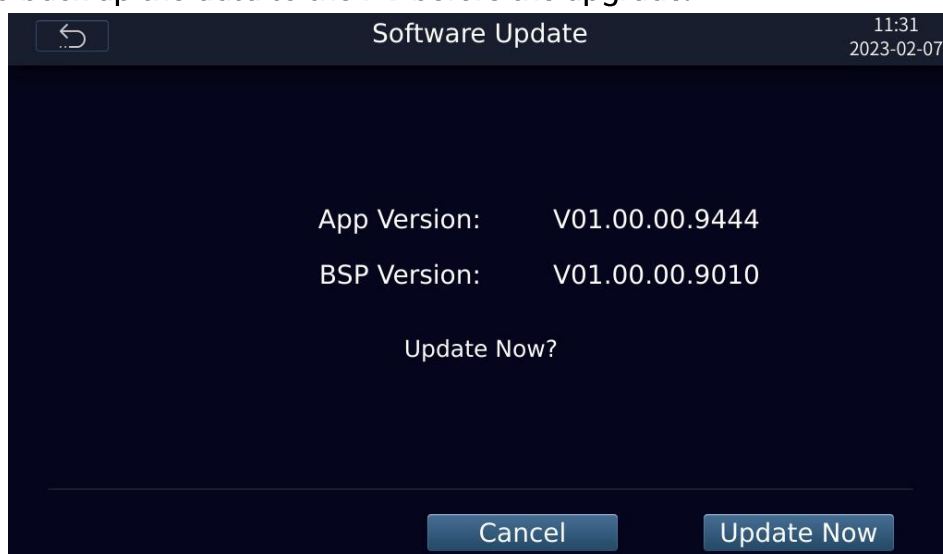


Fig. 7-24

- **Replacement of fluorescence cube**

- 1) First click the [Reset FL Cube] on the [System Setting] page to move the fluorescence cube up. After the reset is successful, please replace the fluorescence cube when turns off the power switch and disconnects the device power.
- 2) Switch off the power switch and power off the device and immediately replace the cube.
- 3) Prepare the new fluorescence cube, loosen the lock screw on rear cover panel with the supplied slotted screw drive counterclockwise, and remove it to show two fluorescence cubes fixed inside the cell counter.

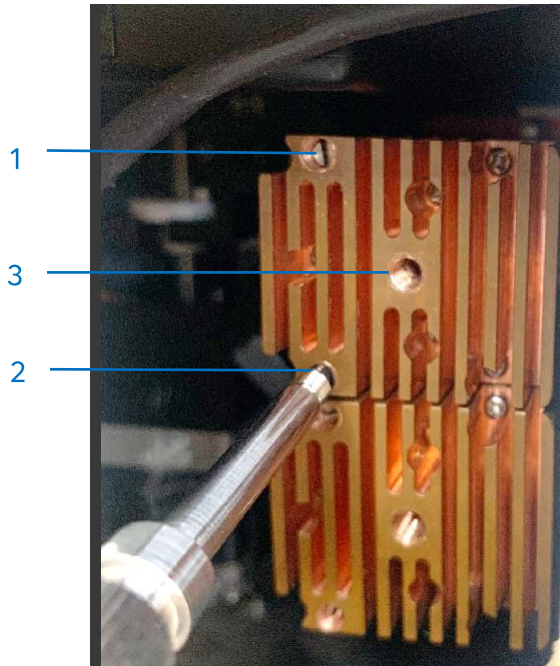


Fig. 7-25

- 4) Take out the screwdriver embedded in the back cover panel;
- 5) Select the fluorescence cube to be replaced, loosen screw 1 and screw 2 on one of the fluorescence cubes counterclockwise, then screw the screwdriver clockwise into hole 3 in the centre of the fluorescence cube until it stops rotating, and then pull out the fluorescence cube and screwdriver horizontally.
- 6) Install the prepared fluorescence cube in the original position and lock it tightly to complete the installation. Install the other cube with the same method.

8- Troubleshooting

Review the information in the table below to troubleshoot operating problems.

For application question, please contact TechnicalProductSupportNA@vwr.com (English only).

Fault/alarm	Solution
Autofocus fails to focus cells well	Make sure there is no bubble or debris on the screen to interfere with autofocus.
Fluorescence is extremely intense and penetrates into other optical filters.	Reduce the RFU before counting.
The USB flash drive failed to be recognized	<ol style="list-style-type: none"> 1) The USB flash drive is not FAT32 format. Format it into FAT32. 2) Replace a USB flash drive in the correct format.
Software update fails	<ol style="list-style-type: none"> 1) Make sure the USB flash drive is FAT32 format, and upload the file to the USB flash drive for software update. 2) The file must be saved into the root directory of the USB flash drive instead of saving it in a folder or subfolder. 3) The file must be intact with the extension .zip during transmission.
Alarm prompt "Motor jammed, please contact the after-sales personnel!" pops up	Power off the device and contact local Avantor services or visit www.vwr.com
Alarm information "FL Cube motor error, FL mode will be disabled" pops up	Power off the device and contact local Avantor services or visit www.vwr.com
Alarm prompt "Camera error, please contact after-sales personnel!"	Power off the device and contact local Avantor services or visit www.vwr.com

9- General maintenance

1) Touch screen cleaning

- Gently and carefully clean the touch screen with a soft lint free cloth moistened in an LCD cleansing detergent. Wipe the touch screen dry immediately after cleaning.
- Prevent ingress of cleaning solvent into the power button, power socket, counting slide port or USB port.
- Never pour or spray any liquid onto the instrument in order to avoid electric shock when the instrument is powered on.
- Never use any corrosive cleaning solution against any scratch on the touch screen.

2) Case cleaning

- Clean the case with a soft lint free cloth moistened in distilled water. Wipe it dry immediately after cleaning.
- Prevent ingress of water or other cleaning solvent into the Power button, power socket, counting slide port or USB port.
- Never pour or spray any liquid onto the instrument in order to avoid electric shock when the instrument is powered on.

3) Decontaminating

- Clean the outer case with a soft lint free cloth moistened in 70% alcohol. Wipe it dry immediately after cleaning.
- Never use a bleach solution that may cause bleaching liquid crystal on the instrument.
- Prevent ingress of water or other cleaning solvent into the power button, power socket, counting slide port or USB port.

Never pour or spray any liquid onto the instrument in order to avoid electric shock when the instrument is powered on.

4) Disposal

When the equipment is removal from use, transportation or disposal, please contact the
Email: TechnicalProductSupportNA@vwr.com.

10- User replaceable accessories, spare parts and compatible dyes

Description	Quantity	Cat. No.
VWR® Fluo Cube Green GFP	1	49893-4951
VWR® Fluo Cube Red RFP-PI	1	49893-4952
VWR® Fluo Cube Blue DAPI	1	49893-4950
AC adapter (NA)	1	/
VWR® Cell counting slides	1 box of 50 pcs	10228-0050
Acridine orange (chlorid salt) 10 mg/ml in water, high purity (AO)	Each(10ml)	89139-110
Propidium iodide 50 ug/mL in PBS	Each(2ml)	89411-112
Hoechst 33342 (bisbenzimidazole H-33342 trihydrochloride) 10 mg/mL in water	Each(10ml)	89139-124
Calcein-AM 4 mM in anhydrous DMSO fluorescent dye, for histology	Each(100ul)	89139-470
Viability/Cytotoxicity Assay Kit for Animal Live and Dead Cells	150 Assays	89260-208

11- Technical service

Visit the VWR website at www.vwr.com for:

- Complete technical service contact information
- Access to the VWR Online Catalogue, and information about accessories and related products
- Additional product information and special offers

Contact us For information or technical assistance contact your local VWR representative or visit www.vwr.com.

12- Warranty

VWR warrants that this product will be free from defects in material and workmanship for a period of two (2) years from date of delivery. If a defect is present, VWR will, at its option and cost, repair, replace, or refund the purchase price of this product to the customer, provided it is returned during the warranty period. This warranty does not apply if the product has been damaged by accident, abuse, misuse, or misapplication, or from ordinary wear and tear. If the required maintenance will not be performed according to the manuals and any local regulations, such warranty turns invalid, except to the extent, the defect of the product is not due to such non-performance.

Items being returned must be insured by the customer against possible damage or loss. This warranty shall be limited to the aforementioned remedies. IT IS EXPRESSLY AGREED THAT THIS WARRANTY WILL BE IN LIEU OF ALL WARRANTIES OF FITNESS AND IN LIEU OF THE WARRANTY OF MERCHANTABILITY.

13- Compliance with local laws and regulations

The customer is responsible for applying for and obtaining the necessary regulatory approvals or other authorisations necessary to run or use the Product in its local environment. VWR will not be held liable for any related omission or for not obtaining the required approval or authorisation, unless any refusal is due to a defect of the product.

Equipment disposal



This equipment is marked with the crossed out wheeled bin symbol to indicate that this equipment must not be disposed of with unsorted waste.

Instead it's your responsibility to correctly dispose of your equipment at lifecycle -end by handling it over to an authorized facility for separate collection and recycling. It's also your responsibility to decontaminate the equipment in case of biological, chemical and/or radiological contamination, so as to protect from health hazards the persons involved in the disposal and recycling of the equipment. For more information about where you can drop off your waste of equipment, please contact your local dealer from whom you originally purchased this equipment.

By doing so, you will help to conserve natural and environmental resources and you will ensure that your equipment is recycled in a manner that protects human health.

Thank you

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